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NSF Engineering Research Center for Biorenewable Chemicals

CBiRC

NSF Engineering Research Center for Biorenewable Chemicals

FOURTH ANNUAL REPORT

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Dr. Basil Nikolau, Deputy Director

Core Partner Institutions

Iowa State University (Lead)

Pennsylvania State University

Rice University

University of California, Irvine

University of New Mexico

University of Virginia

University of Wisconsin, Madison

A National Science Foundation Engineering Research Center since 2008

Cooperative Agreement No. EEC-0813570

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Joseph P. Noel *(Lead)*	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
T1.2 – Acetoacetyl-CoA: Use of *Escherichia coli* for the Production of Molecules Functionalized for Chemical Synthesis		
Thomas A. Bobik *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
T1.3 – Acetyl-CoA/Propionyl-CoA Synthetase: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
David J. Oliver	Genetics, Development & Cell Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
T1.4 – Acyl-CoA Carboxylases: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
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Eran Pichersky *(Lead)*	Molecular, Cellular & Developmental Biology	University of Michigan
Joseph P. Noel	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies

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Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
ASSOCIATED PROJECTS		
A Genetically Tractable Microalgal Platform for Advanced Biofuel Production *U. S. Department of Energy*		
Martin Spalding (*Lead*)	Genetics, Development & Cell Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
David J. Oliver	Genetics, Development & Cell Biology	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics *National Institutes of Health*		
Eve S. Wurtele *(Lead)*	Genetics, Development & Cell Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
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Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Biocatalysts of the Acetyl-CoA Condensation Metabolic Pathway *Iowa State University*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Biosynthesis of Alkamides – Experimental Modeling of a Modular Secondary Metabolic Pathway *National Science Foundation*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
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Thomas A. Bobik *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Functional Genomics of the Biotin Metabolic Network of *Arabidopsis* *Iowa State University*		

Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
Mechanistic and Structural Basis for Plant Metabolic Evolution *Howard Hughes Medical Institute*		
Joseph P. Noel *(Lead)*	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
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Joseph P. Noel *(Lead)*	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
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Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Julie A. Dickerson	Electrical & Computer Engineering	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
Uncovering Novel Signaling Interactions in Plant Metabolic Networks *National Science Foundation*		
Eve S. Wurtele *(Lead)*	Genetics, Development & Cell Biology	Iowa State University

Thrust 2 — Microbial Metabolic Engineering

Faculty Investigators	Department	Institution
CENTER-CONTROLLED (CORE) PROJECTS		
T2.1A – Strain Construction and Optimization in *E. coli*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University

T2.1B – Strain Construction and Optimization in *S. cerevisiae*		
Nancy A. Da Silva (*Lead*)	Chemical Engineering & Materials Science	University of California – Irvine
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
T2.2A – Strain Characterization and Optimization in *E. coli*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
T2.2B – Strain Characterization and Optimization in *S. cerevisiae*		
Nancy A. Da Silva (*Lead*)	Chemical Engineering & Materials Science	University of California – Irvine
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
T2.3A – Omics Experiments in *E. coli*		
Ramon Gonzalez (*Lead*)	Chemical & Biomolecular Engineering	W. M. Rice University
Julie A. Dickerson	Electrical & Computer Engineering	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
T2.3B – Omics Experiments in *S. cerevisiae*		
Laura R. Jarboe (*Lead*)	Chemical & Biological Engineering	Iowa State University
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University

T2.4A – Flux Analysis in *E. coli*		
Jacqueline V. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Costas D. Maranas	Chemical Engineering	Pennsylvania State University
Ka-Yiu San	Bioengineering	W. M. Rice University
T2.4B – Flux Analysis in *S. cerevisiae*		
Jacqueline V. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Costas D. Maranas	Chemical Engineering	Pennsylvania State University
T2.5A – Bioinformatics in *E. coli*		
Julie A. Dickerson (*Lead*)	Electrical & Computer Engineering	Iowa State University
Ramon Gonzalez	Chemical & Biomolecular Engineering	W. M. Rice University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Ka-Yiu San	Bioengineering	W. M. Rice University
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University
T2.5B – Bioinformatics in *S. cerevisiae*		
Eve S. Wurtele (*Lead*)	Genetics, Development & Cell Biology	Iowa State University
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University
T2.6A – Beta-oxidation Pathway Reversal in *E. coli*		
Ramon Gonzalez (*Lead*)	Chemical & Biomolecular Engineering	W. M. Rice University

ASSOCIATED PROJECTS		
A Native Pathway for the Production of n-butanol in *Eschericia coli*: A New Paradigm for Synthetic Biology *National Science Foundation*		
Ramon Gonzalez (*Lead*)	Chemical & Biomolecular Engineering	W. M. Rice University
A Robust Platform for Reconstituting and Engineering Iterative Megasynthases *National Institutes of Health*		
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
CAREER: Understanding and Harnessing the Fermentative Metabolism of Glycerol in *E. coli* – A New Path to Biofuels and Biochemicals *National Science Foundation*		
Ramon Gonzalez *(Lead)*	Chemical & Biomolecular Engineering	W. M. Rice University
Collaborative Research: Metabolic Engineering of Terpenoid Indole Alkaloids Using Transcriptional Regulators in *C. roseus* Hairy Roots *National Science Foundation*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Designed Feedback Regulation of Electron Transport Energetics *National Institutes of Health*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Efficient Synthesis of Hydrocarbons Using an Engineered Reversal of the β-oxidation Cycle: A New Paradigm for the Production of Advanced Biofuels *National Science Foundation*		
Ramon Gonzalez *(Lead)*	Chemical & Biomolecular Engineering	W. M. Rice University
EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels *National Science Foundation*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Thomas A. Bobik	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator *ConocoPhillips Company*		
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University

Engineering an Efficient Biocatalyst for Chiral Compound Production *National Science Foundation*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Engineering Yeast Consortia for Surface-Display of Complex Cellulosome Structure: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol *National Science Foundation*		
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed *Pioneer Hi-Bred International, Inc.*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Fermentative Utilization of Pyrolytic Substrates *National Science Foundation*		
Laura R. Jarboe *(Lead)*	Chemical & Biological Engineering	Iowa State University
Generation of Biofuels from Abundant Non-digestible Oilseed Components *National Science Foundation*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Mass Spectrometric Imaging of Plant Metabolites *U. S. Department of Energy*		
Basil J. Nikolau *(Lead)*	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Metabolic Engineering of *Moritella marinus* to Produce DHA: Transcriptome Sequencing *Metabolic Technologies, Inc.*		
Laura R. Jarboe *(Lead)*	Chemical & Biological Engineering	Iowa State University
MRI: Acquisition of a Tandem (IT-TOF) Mass Spectrometer System for Biological Research and Application *National Science Foundation*		
Ka-Yiu San *(Lead)*	Bioengineering	W. M. Rice University
Use of Systems Biology Approaches to Develop Advanced Biofuel-Synthesizing Cyanobacterial Strains *U.S. Department of Energy*		
Costas D. Maranas *(Lead)*	Chemical Engineering	Pennsylvania State University

Thrust 3 — Chemical Catalyst Design

Faculty Investigators	Department	Institution
CENTER-CONTROLLED (CORE) PROJECTS		
T3.2 – Selective Dehydration of Model Compounds		
Brent H. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
T3.3 – Deoxygenation of Fatty Acids		
Robert J. Davis *(Lead)*	Chemical Engineering	University of Virginia
George A. Kraus	Chemistry	Iowa State University
Matthew Neurock	Chemical Engineering	University of Virginia
L. Keith Woo	Chemistry	Iowa State University
T3.5 – Ring Opening Reactions		
James A. Dumesic *(Lead)*	Chemical Engineering	University of Wisconsin – Madison
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
Robert J. Davis	Chemical Engineering	University of Virginia
Matthew Neurock	Chemical Engineering	University of Virginia
T3.7 – Hydrothermally Stable Catalysts and Catalyst Supports		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
Klaus Schmidt-Rohr	Chemistry	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
T3.9 – Pyrone Conversions		
George A. Kraus (*Lead*)	Chemistry	Iowa State University
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison

T3.10 – Selective Oxidation to Di-acids		
Robert J. Davis *(Lead)*	Chemical Engineering	University of Virginia
Matthew Neurock	Chemical Engineering	University of Virginia
T3.11 – Migration of Functional Groups		
L. Keith Woo (*Lead*)	Chemistry	Iowa State University
SPONSORED PROJECTS		
ERC – Small Business: Commercialization of Furanic-based Biorenewable Chemicals *National Science Foundation*		
Adam L. Okerlund *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
George A. Kraus	Chemistry	Iowa State University
Furanics-based Biorenewable Chemicals *Iowa Board of Regents (Grow Iowa Values Fund)*		
Adam L. Okerlund *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Proprietary Project – Title Undisclosed *Chevron Phillips Chemical Company, LLC*		
Brent H. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Adam L. Okerlund	ERC for Biorenewable Chemicals	Iowa State University
ASSOCIATED PROJECTS		
A Systems Approach to Bio-Oil Stabilization *U. S. Department of Energy*		
Brent H. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Acquisition of X-Ray Diffractometer for Nano-Bio Materials and Earth Sciences Research *National Science Foundation*		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico
GOALI: Understanding Self-assembly of Noble Metal Alloys for Ultra Low Temperature Oxidation Catalysis *National Science Foundation*		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico
Green Catalysis *National Science Foundation*		
L. Keith Woo (*Lead*)	Chemistry	Iowa State University

Materials for Energy Conversion *U. S. Department of Energy*		
Abhaya K. Datye	Chemical & Nuclear Engineering	University of New Mexico
Nanostructured Catalysts for Hydrogen Generation from Renewable Feedstocks *U. S. Department of Energy*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico
National Advanced Biofuels Consortium *U. S. Department of Energy*		
Brent H. Shanks (*Lead*)	Chemical & Biological Engineering	Iowa State University
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials* *National Science Foundation*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico
Ib Chorkendorff	Physics	Technical University of Denmark
Robert J. Davis	Chemical Engineering	University of Virginia
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
George A. Kraus	Chemistry	Iowa State University
Dmitry Murzin	Chemical Engineering	Abo Akademi University
Matthew Neurock	Chemical Engineering	University of Virginia
Hans Niemantsverdriet	Schuit Institute of Catalysis	Eindhoven University of Technology
Robert Schlögl	Inorganic Chemistry	Fritz Haber Institute of the Max Planck Society
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Research Experiences for Undergraduates in Nanoscience and Microsystems *National Science Foundation*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico
Responsive Catalysts *U.S. Department of Energy*		
L. Keith Woo (*Lead*)	Chemistry	Iowa State University

Selective Hydrogenation of Oxygenates *Engineering and Physical Sciences Research Council (United Kingdom)*		
Matthew Neurock	Chemical Engineering	University of Virginia
Selective Oxidation of Polyols *National Science Foundation*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico
Robert J. Davis	Chemical Engineering	University of Virginia
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
Matthew Neurock	Chemical Engineering	University of Virginia
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Structure and Function of Supported Base Catalysts *U. S. Department of Energy*		
Robert J. Davis (*Lead*)	Chemical Engineering	University of Virginia
The Science and Engineering of Durable Ultra-Low Platinum Group Metal Catalysts *Los Alamos National Labs*		
Abhaya K. Datye *(Lead)*	Chemical & Nuclear Engineering	University of New Mexico

Life Cycle Assessment Support Area

Faculty Investigators	Department	Institution
CENTER-CONTROLLED (CORE) PROJECTS		
Techno-Economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars		
Robert P. Anex *(Lead)*	Biological Systems Engineering	University of Wisconsin – Madison
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
ASSOCIATED PROJECTS		
A Regional Program for Production of Multiple Agricultural Feedstocks and Processing to Biofuels and Biobased Chemicals *U.S. Department of Agriculture*		
Robert P. Anex	Biological Systems Engineering	University of Wisconsin – Madison

Pre-College Education

Faculty Investigators	Department	Institution
CENTER-CONTROLLED (CORE) PROJECTS		
Teacher Professional Development (RET and Summer Academy Programs)		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Pre-College Learning Modules		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
Young Engineers Program		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Joseph P. Noel	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
SPONSORED PROJECTS		
Integration of STEM through Problem-based Learning[1] *Des Moines Public Schools*		
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University
***Symbi*, Iowa's GK-12 Program: Growing Iowa's Scientists for a Greener Tomorrow** *National Science Foundation*		
Adah Leshem *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
ASSOCIATED PROJECTS		
Enhancing Energy Education in Iowa *Office of Energy Independence, State of Iowa*		
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University

Meta!Blast: An Immersive Interactive Learning Module for Cell Biology *National Institutes of Health*		
Eve S. Wurtele (*Lead*)	Genetics, Development & Cell Biology	Iowa State University
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University
Plants in Society *National Science Foundation*		
Adah Leshem	ERC for Biorenewable Chemicals	Iowa State University

University Education

Faculty Investigators	Department	Institution
CENTER-CONTROLLED (CORE) PROJECTS		
CBiRC Graduate Minor and Graduate Certificate Programs		
D. Raj Raman *(Lead)*	Agricultural & Biosystems Engineering	Iowa State University
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
CBiRC Research Experience for Undergraduates (REU) Program		
D. Raj Raman *(Lead)*	Agricultural & Biosystems Engineering	Iowa State University
Robert P. Anex	Biological Systems Engineering	University of Wisconsin – Madison
Nancy A. Da Silva	Chemical Engineering & Materials Science	University of California – Irvine
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
Laura R. Jarboe	Chemical & Biological Engineering	Iowa State University
George A. Kraus	Chemistry	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Joseph P. Noel	Jack H. Skirball Center for Chemical Biology & Proteomics	Salk Institute for Biological Studies
Peter J. Reilly	Chemical & Biological Engineering	Iowa State University
Derrick Rollins	Chemical & Biological Engineering / Statistics	Iowa State University
Suzanne B. Sandmeyer	Biological Chemistry	University of California – Irvine

Brent H. Shanks	Chemical & Biological Engineering	Iowa State University
Jacqueline V. Shanks	Chemical & Biological Engineering	Iowa State University
L. Keith Woo	Chemistry	Iowa State University
Eve S. Wurtele	Genetics, Development & Cell Biology	Iowa State University
SPONSORED PROJECTS		
EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels *(REU Supplement)*[2] *National Science Foundation*		
Jacqueline V. Shanks *(Lead)*	Chemical & Biological Engineering	Iowa State University
Thomas A. Bobik	Biochemistry, Biophysics & Molecular Biology	Iowa State University
Basil J. Nikolau	Biochemistry, Biophysics & Molecular Biology	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
Iowa State Coleman Faculty Entrepreneurship Fellow: Development of BR C 507X, *Entrepreneurship in Biorenewable Chemicals* *Coleman Foundation (channeled through the ISU Foundation)*		
Peter L. Keeling *(Lead)*	ERC for Biorenewable Chemicals	Iowa State University
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University
ASSOCIATED PROJECTS		
A Virtual Education Center for Biorenewable Resources: Building Human Capital and Humanizing Distance Education *U. S. Department of Agriculture*		
D. Raj Raman	Agricultural & Biosystems Engineering	Iowa State University

International Education

Faculty Investigators	Department	Institution
ASSOCIATED PROJECTS		
PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials[3] *National Science Foundation*		
Abhaya K. Datye (*Lead*)	Chemical & Nuclear Engineering	University of New Mexico

Ib Chorkendorff	Physics	Technical University of Denmark
Robert J. Davis	Chemical Engineering	University of Virginia
James A. Dumesic	Chemical Engineering	University of Wisconsin – Madison
George A. Kraus	Chemistry	Iowa State University
Dmitry Murzin	Chemical Engineering	Abo Akademi University
Matthew Neurock	Chemical Engineering	University of Virginia
Hans Niemantsverdriet	Schuit Institute of Catalysis	Eindhoven University of Technology
Robert Schlögl	Inorganic Chemistry	Fritz Haber Institute of the Max Planck Society
Brent H. Shanks	Chemical & Biological Engineering	Iowa State University

Footnotes:

1. See Project Summary entitled "Teacher Professional Development" under Pre-College Education.
2. See Project Summary entitled "Research Experience for Undergraduate (REU) Program" under University Education Program.
3. See Project Summary entitled "PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials," under International Education Program.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.1 – 3-Ketoacyl-ACP Synthase:** Characterization of Novel Biocatalysts for Diversifying FAS/PKS Metabolic Pathways

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Joseph P. Noel	**02/17/2012**	**9/28/09 to 02/29/2012**

ERC Team Members

Project Leader: Joseph P. Noel, Salk Institute for Biological Studies
Other Faculty: Basil J. Nikolau, Peter J. Reilly, Iowa State University; Eran Pichersky, University of Michigan
Postdocs: Kate Woods, Charles Stewart and Yongxia Guo, Salk Institute
Graduate Students: David Cantú, Yingfei Chen, Huanan Jin and Shivani Garg, Iowa State University
Undergraduate Students: Bing O'Dowd (University of California, San Diego), Adam Dobson (University of Pittsburgh), Aidan Gilchrist (University of Massachusetts Amherst), Phillip Guichet (University Of San Diego), Erina He (University Of California, San Diego), Kyle Merchant (University of California, San Diego), Salk Institute, and Morgan Becker, Iowa State University
High School Students: Alyssa Herperger (Canyon Crest Academy), Salk Institute
Other Personnel: Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

The overarching goal of this project is to identify and characterize novel biocatalysts from plant and microbial polyketide synthase (PKS) systems for the purpose of diversifying the malonyl-CoA pools of *E. coli* and the yeast *Saccharomyces cerevisiae*. This project targets biocatalysts that will expand substrates used in the carbon-carbon and carbon-oxygen bond forming reactions of fatty acid and polyketide biosynthetic cycles. Specifically, we focus on (1) the 3-ketoacyl-ACP synthase III (KAS III) family of condensing enzymes, (2) the evolutionarily related type III PKSs commonly found in plants and (3) the iterative type I FAS–type III PKS hybrid megasynthases from *Dictyostelium discoideum* known as Steelys. The objective of project goal 1 is to clone, express, biochemically characterize, and when successful, crystallize and solve the atomic resolution 3D structures of orthologs of KAS IIIs from hosts with unconventional substrate selection (acetyl-CoA being conventional), to create new metabolic intermediates for enzymes examined as parts of goals 2 and 3. The objective of project goal 2 is to create a platform to evaluate structure-based mutant libraries of *Gerbera hybrida* 2-pyrone synthase (2-PS) and evolutionarily related plant type III polyketide synthases (PKS IIIs) such as chalcone, stilbene, orcinol and bibenzyl synthases that employ one molecule of acetyl-CoA and two molecules of malonyl-CoA to biosynthesize 6-methyl-4-hydroxy-2-pyrone (2-PY), one of our lead test beds, or orcinol (5-methylbenzene-1,3-diol), a developing test bed. Given our

atomic resolution structural knowledge of 2-PS and related type IIIs, we will create mutant libraries centered on the active site (focused) and random libraries spread throughout the protein to uncover catalytically more efficient enzymes for 2-PY production. A second objective is to employ rationally designed fusion proteins of type III PKSs with the biotin-containing subunit of heterotetrameric acetyl-CoA carboxylases to greatly increase the local concentration of malonyl-CoA near the targeted PKS. The objective of project goal 3 is to employ a microbially optimized synthetic gene encoding *Dictyostelium discoideum* Steely 2 and mutants thereof to produce short chain saturated fatty acid products and short chain unsaturated fatty acid products (~6 carbons in length based upon the known product of Steely 2) as well as pyrones.

Using a combination of atomic resolution protein x-ray crystallography, site-directed and combinatorial mutagenesis and high-throughput in vitro biochemistry, we will rationally modulate the efficiency and specificity of all project goal targets for the production of short-chain keto-containing products for downstream processing or as test bed end products. By the end of Year 4, our goal is to engineer at least two biocatalysts that efficiently produce a reactive intermediate or end product in a microbial fermentation system (Thrust 2) that, upon scale-up and isolation, is delivered to Thrust 3 for large-scale chemical processing.

The Reilly group will construct databases of all 3-ketoacyl synthase genes, proteins and structures that appear in the literature and in public databases. Based on the phylogenetic analysis of KASIII done by the Reilly group, the Nikolau group will clone, express and functionally characterize KASIII genes from diverse sources with distinct substrate specificities as a part of goal 1. Nikolau group will also develop a high-throughput genetic screen to identify KASIII enzymes capable of synthesizing branched chain fatty acids. The Noel group will in addition to carrying out the structure-based mutagenesis and biochemical characterization of enzymes as part of goals 2-3, will carry out protein x-ray crystallographic structure determination of key mutants uncovered as part of goals 2-3 for evaluation by the Reilly group.

Project's Role in Center's Strategic Plan

A diverse collection of KASIII enzymes occurs in different biological systems that utilize different acyl-CoA substrates in this reaction. These ultimately add functionalities at the omega-end of the fatty acid products. The goal of this project is two-fold: 1) Find and characterize the molecular details of the nature of these KASIII orthologs that display different substrate specificities; and 2) based on the understanding of the design principle of these KASIII enzymes, create by mutagenesis novel KASIII orthologs that display distinct substrate specificities.

The atomic resolution crystal structure of the *G. hybrida* 2-PS was determined in the Noel lab. The structure confirmed the validity of an homology model's active site predictions by revealing the active site cavity of 2-PS to possess only a third of the volume observed in a previously determined x-ray structure of a related enzyme, chalcone synthase (CHS), that employs larger starter molecules and 3 molecules of malonyl-CoA for the iterative production of chalcone. Most significantly, mutation of three CHS active site cavity residues to their 2-PS counterparts is sufficient to make alfalfa CHS functionally identical, both in terms of specificity and kinetics, to 2-PS. The remarkable functional conversion of CHS to 2-PS by changing less than 1% of their differing residues supports an intuitively simple model of the steric modulation hypothesis, thus, setting the stage for the structure-based approaches integral to the goals of this Thrust 1 project.

Although no CHS-like PKS IIIs were originally known outside of plants and bacteria, we discovered two putative type III PKS sequences with conservation of key catalytic and structural residues (Steely) in slime molds. Further analysis of the surrounding genomic environment unexpectedly revealed that these two CHS-like sequences comprise the C-terminal domains of two ~3,000-residue predicted megaproteins with significant sequence and domain homology to mammalian iterative type I FASs. Except for substitution of the normally expected C-terminal product-releasing thioesterase (TE) domain with CHS-like domains, these predicted hybrid megasynthases otherwise conserved the mammalian FAS-like domain arrangement, including conservation of important catalytic residues for each FAS enzymatic domain. Analogy with type I FAS and PKS systems suggests the covalent transfer of N-terminal FAS acyl thioester intermediate directly from the post-translationally added phosphopantetheine prosthetic arm of the upstream ACP domain to the active site Cys of the juxtaposed C-terminal CHS-like domain. Together, these results confirm not only the function but also the efficient molecular logic suggested by the domain organization of the novel Steely hybrid megasynthases, thus providing an evolutionarily optimized template for engineering other desirable hybrid type I/type III PKS pathways.

Fundamental Barriers and Methodologies

- Development of specific substrates (e.g. malonyl ACP) for the KAS III spectrophotometric enzymatic assay is a bottleneck due to their poor stability.
- The unavailability of KAS III gene sequences from organisms that synthesize halogenated fatty acids has limited the work to KAS III sequences from organisms that make branched chain or hydroxy-fatty acids.
- Lack of more catalytically efficient PKS IIIs for the practical production of the pyrone test bed products integral to the aims of Thrusts 1-3.
- Low level expression of the Steely 2 megasynthase capable of high -level protein and small molecule product (acylphloroglucinols, pyrones and short chain fatty acids) production in microbial hosts.

Achievements

Prior to 2/28/2011:

- Optimized the expression and purification of recombinant KAS IIIs. Specifically, *E. coli* KASIII (i.e. FabH), *B. subtilis* KASIIIa (i.e. YjaX) and *B. subtilis* KASIIIb (i.e., YhfB) proteins. Obtained yields in the range of 10-20 mg/L of *E. coli* culture.
- Conducted spectroscopic and physical characterizations of recombinant *E. coli* KASIII, *B. subtilis* KASIIIa, KASIIIb to ensure correct functional folding. CD and 1-D NMR analyses demonstrate that the three proteins are in folded states after purification.
- Via homology modeling using the *E. coli, S. pneumoniae* (Lonsdale et al., 2001) and *S. aureus* (Appelt et al., 2009) KASIII structures as templates we identified structural differences in a specific phenylalanine residue, which is orientated converse in the active site of *B. subtilis* KASIIIa and KASIIIb as compared to *E. coli* KASIII. We predicted that changing the orientation of this Phe residue will contribute to different substrate specificities of these enzymes (i.e., *E. coli* KASIII prefers a straight chain acyl-CoA substrate, whereas *B. subtilis* KASIIIa and KASIIIb prefer branched chain acyl-CoA substrates). To test this hypothesis we generated site-directed mutants of *E. coli* KASIII and *B. subtilis* KASIIIa and KASIIIb, in which the mutated

residues are predicted to change the orientation of the key Phe residue (See current achievements, below).

- An understanding of the structural basis of differences in substrate specificities of *E. coli* KASIII as compared to *B. subtilis* KASIIIa and KASIIIb has been achieved using Saturation Transfer Difference (STD) NMR. STD NMR was used to study the binding of wild-type and double mutant KASIII enzymes (*E. coli* KASIII and *B. subtilis* KASIIIa) with two different ligands (i.e. straight chain acetyl CoA and branched chain isobutyryl CoA). Our data indicated that the simultaneous mutation of two residues in *E. coli* KASIII to the corresponding residues in *B. subtilis* KASIIIa affect the orientation of the key phenylalanine, which in turn makes *E. coli* KASIII capable of interacting with a branched chain ligand. Whereas, simultaneous mutation of the same residues in *B. subtilis* KASIIIa to the corresponding residues in *E. coli* KASIII results in loss of its ability to bind with a branched chain ligand. The effect of mutating each of the residues separately (i.e. single mutants) is discussed in the current achievements section below.
- Development of a working high-throughput enzymatic assay (currently being optimized) to determine the activities of diverse KASIII enzymes, including *E. coli* KASIII, *B. subtilis* KASIIIa and KASIIIb.
- Computational classification of Ketoacyl Synthases (KS) into five families based on similarities in their primary sequences and tertiary structures (Reilly group). Sequences have been deposited in the ThYme database (www.enzyme.cbirc.iastate.edu).
- Construction of a phylogenetic tree containing >2000 KAS III sequences that sorted into 12 unique subfamilies within KS family #1.
- Selection of 30 diverse KASIII genes from the phylogenetic classification, and chemically synthesized gene sequences (codon optimized for *E. coli*) for the purpose of expressing them and characterizing their properties in supporting fatty acid biosynthesis *in vivo*. Genes were selected such that 1) each subfamily was represented; 2) multiple KAS genes from a single organism in more than one subfamily were represented; and 3) they came from organisms that are known to produce branched chain or hydroxy fatty acids.
- Generation of a *B. subtilis* recipient strains for testing KASIII function. In these strains either one or both of the *Bacillus* KASIII homologs (KASIIIa, KASIIIb) have been knocked out. Fatty acid profiling showed that KASIIIa has a higher activity toward isovaleryl CoA whereas KASIIIb has higher preference for isobutyryl CoA and acetyl CoA. Both share similar activity toward anteisovaleryl CoA. Moreover, because the double knockout *Bacillus* strain is lethal and can be genetically rescued by the expression of KASIII homologs, this strain is an ideal recipient strain for the *in vivo* testing of KASIII homologs.
- A high throughput assay for mutant characterization including two automated robotic platforms for mutant protein isolation and assay development are in place to support all project goals.
- A spectrophotometric assay has been developed to follow the in vitro production of pyrones allowing high-throughput analysis of PKS III mutant libraries in part using the robotic platform just mentioned.
- Using a combination of host codon optimization for yeast and *E. coli* along with appropriate translational pause sites for proper folding of these megasynthases, a synthetic gene for Steely 1 has been constructed and delivered to Thrust 2 for initial analysis in microbial hosts.
- 2-PS and related type III PKSs such as CHS, while having a primary polyketide forming activity,

also efficiently decarboxylate malonyl-CoA to acetyl-CoA. This observation drives optimization of 2-PS's 2PY forming activity to increase turnover number for 2-PS, and temper efficient decarboxylation of malonyl-CoA prior to initial loading of an acetyl moiety.

- Two orthogonal assays have been optimized and a third is under development. First, in order to screen mutants quickly for the production of 2-PY, a simple absorbance based assay is used, screening using a defined mixture of both 2-PS substrates acetyl-CoA (initiator) and malonyl-CoA(extender)and can be performed in a 96 or 384 well format.
- An NMR based assay was developed to precisely track carbon flow during the course of type III PKS reactions employing ^{13}C-malonyl-CoA and/or ^{13}C-acetyl-CoA.
- Identification of an oxidation-mediated self-regulatory mechanism focused on a key catalytic Cys residue was discovered in plant type III PKSs.
- A means to counteract this oxidation event leading to diminished iterative carbon-carbon bond forming activity was identified by expanding our phylogenetic analysis of type III PKSs in the green plant lineage and in microbial lineages. We discovered a CHS from a basal taxa, *Selaginella* where the active site cysteine does not undergo oxidation. Mutations known to switch CHS specificity to that of 2-PS were produced in this basal CHS family member.
- A number of more efficient 2-PS point mutants have been discovered that increase turnover by 1.5-2.5-fold.

From 3/1/2011 to 2/29/2012:

- To probe the effects of specific residues in determining substrate specificity of *E. coli* KASIII (i.e. FabH) and *B. subtilis* KASIIIa (i.e. YjaX), we used homology modeling to predict two particular residues that could potentially affect the orientation of Phe304 (and therefore substrate specificity) in *E. coli* KASIII, and Phe297 in *B. subtilis* KASIIIa (Figure 1). Single mutants were constructed for both of these enzymes (Val215Phe or Leu220Met for *E. coli* KASIII, and Phe208Val or Met213Leu for *B. subtilis* KASIIIa).

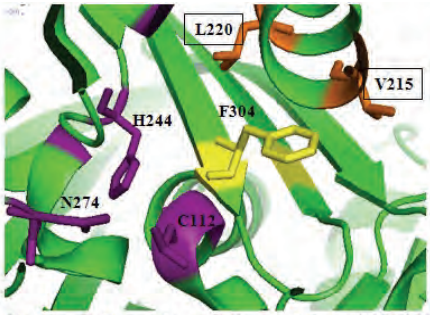

Fig. 1a Active site residues of *E. coli* KASIII (PDB file - 3IL9). Residues C112, N274 and H244 (in magenta) are the active site residues and F304 is proposed to be affecting the substrate specificity, based on its orientation owing to V215 and L220 in the layer behind it.

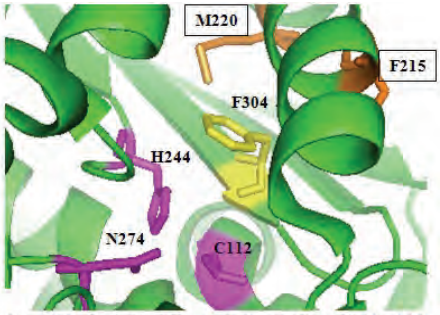

Fig. 1b Proposed model of *E. coli* KASIII after site-directed mutagenesis (V215F and L220M). Note that the rotamer conformation of F304 is different here because of changes in the residues behind it. This should result in change in specificity of the KASIII enzyme.

Interactions of these mutant and wild-type enzymes with straight chain substrate (acetyl CoA) and branched chain substrate (isobutyryl CoA) were studied using STD NMR. STD amplification factors (indicators of ligand binding) from these single mutants were compared with those from the wild-type and double mutant KASIII enzymes from *E. coli* and *B. subtilis*, which we had constructed last year (see previous achievements, above). Comparison of the STD amplification factors revealed that the orientation of Phe297 is affected by the Met213Leu and to a lesser extent, by Phe208Val mutations in *B. subtilis* KASIIIa (Figure 2), resulting in loss of binding with branched chain substrate. In *E. coli* KASIII, the Leu220Met and to a lesser extent, Val215Phe

mutations allow for broader substrate specificity with branched chain substrate in addition to straight chain substrate (Figure 2), possibly by affecting the orientation of Phe304. Together, these data support our hypothesis that Phe304 in *E. coli* KASIII (Phe297 in *B. subtilis* KASIIIa) is one of the critical residues for substrate recognition, and its orientation is impacted by Leu220 and Val215 in *E. coli* KASIII, and by Met213 and Phe208 in *B. subtilis* KASIIIa.

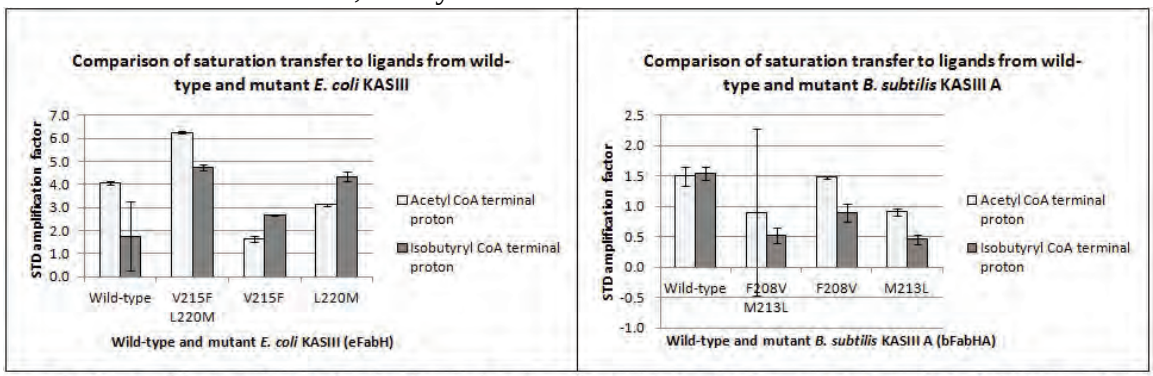

Fig. 2 STD amplification factors for the terminal proton of ligands (acetyl CoA and isobutyryl CoA) based on their interaction with wild-type and mutant *E. coli* and *B. subtilis* KASIII A enzymes – STD amplification factor increases in *E. coli* KASIII V215F L220M mutant, and decreases in *B. subtilis* KASIII A F208V M213L mutant as compared to their respective wild-type enzymes. L220 in *E. coli* KASIII and M213 in *B. subtilis* KASIII A seem to be critical for ligand binding.

Refer to table 1 that summarizes the effects of each of the mutations on substrate specificities of the enzymes.

- A total of 30 KASIII genes were selected and synthesized based on last year's phylogenetic analysis conducted by Dr. Reilly's group. Seven genes (one each from *Acidophilus acidocaldarius, Bacteroides vulgatus, Renibacterium salmonarum* and four from *Legionella pneumophila*) were cloned and expressed in *E. coli*. We were able to purify all but *R. salmonarum* KASIII and two of the *L. pneumophila* KASIIIs. Yields ranging from 1.5-5mg/liter were obtained.
- Purified KASIII enzymes were assayed for their activities with 3-hydroxy butyryl CoA, using the spectrophotometric assay we developed last year. KASIII from *A. acidocaldarius* and *B. vulgatus* showed activity with hydroxylated substrates, in addition to their activities with straight chain and branched chain substrates.
- Preliminary activity assays with two KASIIIs from the same strain of *Legionella pneumophila* indicate that one of them shows specificity for a hydroxylated substrate whereas the other one does not.
- KASIII from *A. acidocaldarius* was co-expressed with *R. rubrum* ketothiolase and acetoacetyl CoA reductase in *R. rubrum*; these two latter enzymes can produce 3-hydroxybutyryl-CoA. Fatty acid profiling showed formation of omega-2 hydroxy fatty acids in *R. rubrum* that are not naturally produced by this organism.
- The KS2 (340 sequences), KS3 (9552 sequences), KS4 (1210 sequences), and KS5 (696 sequences) families contain 10, 14, 10 and 11 subfamilies, respectively. KS2 mainly consists of plant sequences, KS3 consists of sequences from diverse phyla, KS4 has bacterial and plant sequences, whereas KS5 is composed of animal, fungi and protist sequences.
- The NMR based assay has been extended to tracking carbon flow between $^{13}C_4$ -acetoacetyl-CoA (a reaction intermediate) and $^{13}C_3$-malonyl-CoA (the extender).
- The development of a screening method that employs temperature dependent fluorescence to

assess to 2-PS' (and related mutants) relative affinity for different substrate acyl-CoAs that are relevant to the formation of 2-PY by 2-PS (malonyl-CoA, acetyl-CoA and acetoacetyl-CoA), CoASH (a product of the 2-PY formation reaction), and benzoyl-CoA (an alternative initiator acyl-CoA accepted by native 2-PS). We have hypothesized that the area within the 2-PS active site that allows specific acyl starter groups is 'too big', because 2-PS accepts benzoyl-CoA in addition to the much smaller acetyl-CoA. Moreover, structures of 2-PS that have been collected with and without acyl-CoA substrates show a broad binding pocket where the loaded acyl group is proposed to be situated when bound to the active site cysteine prior to binding of malonyl CoA for elongation. By reducing the volume of this pocket, mutants of 2-PS should no longer accept benzoyl-CoA (or show significantly reduced affinity for benzoyl-CoA) and have heightened affinity and/or specificity for acetyl-CoA and acetoacetyl-CoA.

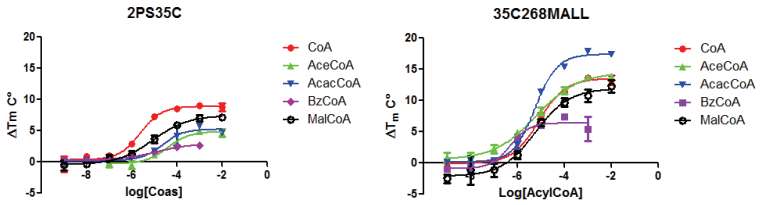

Fig 1: Relative affinity of native 2PS (left) and mutant with 15-fold increase in 2PY production (right) for Coenzyme A (CoASH), acetyl-CoA (AceCoA), Acetoacetyl-CoA (AcacCoA), Benzoyl-CoA (Bz-CoA) and Malonyl-CoA (Mal-CoA). Note that the mutant enzyme shows a marked increase in affinity for Acac-CoA.

- Identification of two key active site positions and amino acid residue substitutions which result in increased specificity for acetoacetyl-CoA and a 10-15 fold increase in turnover in 2-PY in enzymatic assays.
- Identification of a non-active site position in 2-PS, situated on the exterior of the protein, that may be mutated to a residue less prone to oxidation, extending the life of the mutant when expressed in *Saccharomyces cerevisiae* and resulting in increased production of 2-PY over the course of multiple days.
- Development of an enzymatic radiochemical assay using ^{14}C labeled malonyl CoA which allows for accurate detection of smaller amounts of 2-PY produced by 2-PS and related mutants. This is complementary to the spectrophotometric assays currently employed to study these proteins because it allows collection of shorter time points with lower concentrations of acyl-CoA substrates, which is necessary in order to collect steady state kinetic parameters.
- Cloned the first plant orcinol synthase (OS) (plant type III PKS) from the flowers of apricot trees, that like 2-PS, employs acetyl-CoA and two malonyl-CoA molecules for chain initiation and elongation but unlike 2-PS which terminates chain extension through a lactonization reaction, OS terminates elongation using a carbon-carbon bond forming Claisen condensation.

Other Relevant Work

Ketoacyl-ACP synthases from other organisms are being studied by other groups outside the ERC. For example, the structures of FabH from *S. aureus* and *S. pneumoniae* have been determined and proven useful in homology modeling. Those projects are focused on understanding the structure of these enzymes and developing antibiotics that can target these enzymes, which form a critical part of FAS system in bacteria. However, this project on KAS in the ERC not only aims at understanding the structural basis for differences in substrate specificities of KAS enzymes, but also aims at modifying their structures to yield novel biocatalysts useful for the chemical industry.

Plans for the Next Year

- Biophysical and enzymological characterization of 23 diverse KASIII enzymes identified by phylogenetic analyses.
- Determination of activity of cloned KASIII enzymes with non-endogenous substrates such as hydroxyl acyl CoA (e.g. 3-hydroxy butyryl CoA) and aromatic acyl CoA (e.g. benzoyl CoA).
- Identify additional key residues that are responsible for substrate specificities of KASIII enzymes: possible structure determination and mutagenesis (site-directed or random) of interesting candidates to create novel KASIII orthologs with ability to make substituted fatty acids.
- Expression of KASIII genes from diverse sources in the *B. subtilis* KASIII deletion double mutant followed by analysis of substrate preference and fatty acid profiles of these constructs.
- Structure determination of mutants of 2-PS by biophysical methods (e.g., x-ray crystallography).
- Expansion and recombination of more efficient 2-PS mutants to increase 2-PY production via an increase in kcat or a decrease in the non-productive malonyl CoA decarboxylation reaction.
- Parallel development of minimally 5 different plant type III PKSs by rational mutagenesis to transplant 2-PY forming activity into an evolutionarily related enzyme fold.
- Development of an efficient orcinol synthase (OS) from the clone obtained thus far (Noel-Pichersky) that employs the same substrates as 2-PS but terminates its iterative reaction through a ring closing Aldol reaction instead of the carbon-oxygen forming lactonization reaction of 2-PS.

Expected Milestones and Deliverables

- KASIII enzymes with modified substrate specificities that can make carboxylic acids with different chain functional groups, which will have applications as biorenewable chemicals.
- *B. subtilis* KASIII deletion mutant-based genetic screen for high-throughput screening of KASIII enzymes.
- Phylogenetic trees for all five KS families.
- 2-PSs, derived from either mutagenesis of authentic *Gerbera hybrid* 2-PS or the appropriate type III PKS mutants, capable of supporting high yields of pyrone test bed products.
- Kinetic characterization of orcinol synthase (OS), its x-ray structure determination and its stability assesment to expand our polyketide test beds based upon type III PKSs and the simple substrates, acetyl-CoA and malonyl-CoA.
- Dissection of residue differences between OS, 2-PS and bibenzyl synthase (BBS) that dictate Claisen, lactonization and aldol chain-terminating chemistry to offload products.
- Interconversion of OS, 2-PS and BBS activities by site-directed multi-site mutagenesis.

Member Company Benefits

Milestones and deliverables obtained as part of this project should provide small molecule end products including pyrones, short chain fatty acids, and downstream orcinols, that are integral test beds for Thrust 3. Moreover, the KAS III enzymes will provide metabolic intermediates integral to the aims of all projects associated with Thrust 1.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.2 – Acetoacetyl-COA:** Use of *Escherichia coli* for the production of molecules functionalized for chemical synthesis.

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Thomas A Bobik	**2/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Thomas A Bobik**,** Iowa State University
Postdoc: David Gogerty, Iowa State University
Other Personnel: Christian Bartholomay - Assistant Scientist II, Iowa State University

Statement of Project Goals

A major project goal is to develop new enzyme catalysts that allow the efficient conversion of glucose to short-chain carboxylic acids and other molecules shown in figure 1 using genetically engineered *Escherichia coli*. Work will focus on production of hexanoic acid as well as bifunctional C6 compounds. To our knowledge, the production of C6 carboxyolic acids from coenzyme A intermediates has not been reported in the literature. Development of an organism that produces high levels of hexanoyl-CoA will provide a platform for production of all the compounds shown in figure 1. In addition, the molecules shown in figure 1 will be further diversified as new catalysts become available and may also include alkanes, alkenes, α-olefins and methylketones. It will also be necessary to identify enzymes that efficiently release the coenzyme A to produce the desired compound. These catalysts must not cross-react with other CoA derivatives or undesired co-products may be formed. Enzymes used for CoA removal will include acyl-CoA reductases, CoA transferases, thioesterases and methylketone synthases. Highly specific enzymes will be identified.

A second major goal of the project is to develop catalytic systems that allow the production molecules more oxidized than glucose. This requires a means for eliminating excess electrons. In general, others researchers have accomplished this by using aerobic systems where electrons are consumed by the reduction of oxygen to water. In one case, an aerobic system has been modified to minimize carbon loss through the TCA cycle (1). We will use similar approaches, and also develop an alternative anaerobic system that eliminates excess electron by co-production of hydrogen gas. This approach that has some potential advantages. H_2 has a number of industrial uses and the introduction of oxygen into fermentation systems requires a high energy input; hence hydrogen co-production will provide a valuable co-product and may reduce process energy costs. To develop such systems, we will initially co-produce hydrogen and acetaldehyde from glucose. Both compounds have commercial application in the synthesis of industrial chemicals and acetaldehyde is produced from acetyl-CoA which the precursor of all the chemicals in figure 1. Thus, the systems developed for co-production of hydrogen with acetaldehyde will have potential application to production of all the relatively oxidized molecules in figure 1.

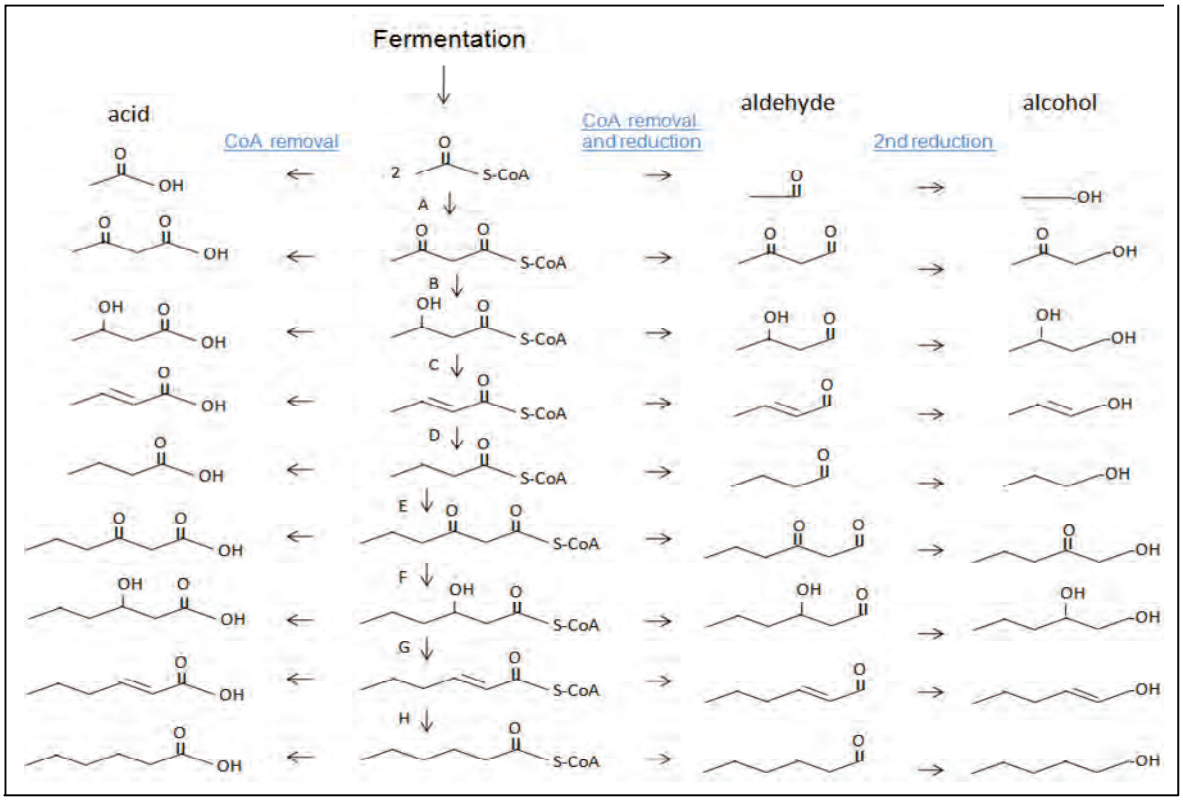

Figure 1. Pathways for the production of renewable chemicals from glucose in *E. coli*.

Project's Role in Center's Strategic Plan

The catalysts used to produce the molecules shown in figure 1 will be used by thrust 2 to develop strains of *E. coli* that efficiently produce large amounts of these chemicals. In turn, these compounds will be used in thrust 3 as platform chemicals for the development of catalyst systems that allow the synthesis of important industrial chemicals. For example, thrust 3 has developed methods for the decarboxylation of organic acids (such as hexanoic acid) to alpha-olefins. Thrust 3 will also develop methods for the production of important polymers from bifunctional molecules such as 3-hydroxyhexanoic acid which also is a target of this project.

Fundamental Barriers and Methodologies

Other groups have produced 1-butanol and 3-hydroxybutyrate by the pathway shown in figure 1. In these systems, the crotonyl-CoA reductases have been problematic. Many are integral membrane proteins that couple to electron transport flavoproteins EftA and EftB and have low activity in *E. coli*. Hence, this enzymatic step is thought to limit 1-butanol production. This same problem is likely to apply to the production of C6 compounds by the pathway of Figure 1. To eliminate this problem, we will use NADH-dependent enoyl-CoA reductase from *Euglena*. Other groups recently reported the efficacy of this method. We have cloned and expressed this enzyme which has high activity in *E. coli*. A second problem in 1-butanol production is its toxicity. However, butanol is not a target compound for this project. The toxicity of our potential targets will be investigated by thrust 2 as they work toward high-level production and will be ameliorated by strain optimization. Three research groups have worked on the production of R- and S-3-hydroxybutyrate as starter molecule for chiral synthesis. Thus far, productivity in these systems is low but encouraging. The main barriers for this system have not yet been clearly identified but are most likely inefficiencies due to the use of a non-optimized aerobic process. Co-production of hydrogen and/or modification of aerobic metabolism will be needed to improve these processes and we are working on both of these approaches.

As mentioned above this project will need to identify catalysts must not cross-react with other CoA derivatives or undesired co-products may be formed. Initially we will focus on carboxylic acid production using appropriate thioesterases. Enzyme with the needed specificity will be identified using one or more of the following approaches: 1. literature searches; 2.screening evolutionarily diverse enzyme identified with the CBiRC developed thioesterase database; rational engineering of enzyme active sites.

Additional problems in metabolic pathway engineering are imbalances in the expression of genes in the engineered pathway which lead to bottlenecks and metabolite/cofactor imbalances that inhibit growth of the producer organism and/or product formation. Balancing gene expression will be done in an iterative fashion based on analysis of product profiles. Improved flux will also be addressed by developing screens for improved production following genetic modification as well as metabolic flux analysis and omics approaches. However, these tasks are mainly the responsibility of Thrust 2.

Other general barriers to this project include (i) the production of active heterologous enzymes with the proper substrate specificity in *E. coli (ii)* the identification of currently unknown catalysts and (iii) maintenance of proper redox balance without the production of undesired co-products. Expression of heterologous enzyme requires a number of considerations including RNA stability, protein solubility, protein toxicity and post-translational regulation. Problems associated with production of active enzymes will be addressed by using gene synthesis in conjunction with computer programs that optimize codon bias, address RNA folding and stability. Development of the needed catalyst specificity will require biochemical and in vivo characterization followed by catalyst evolution if necessary. The main consideration for the identification of new catalysts is the development of efficient high-throughput screening methods and the development of appropriate screens.

Achievements

We have cloned, expressed and purified enzymes that convert acetyl-CoA to butyryl-CoA (reactions A-D in figure 1). These include acetoacetyl-CoA synthase, acetoacetyl-CoA reductase and crotonase from *Clostridium* as well as crotonyl-CoA reductase (CCR) was from *Euglena*. The Euglena CCR is an NADH-dependent enzyme and is expected to give better results than enzymes that use EtfAB as electron acceptor. The purified enzymes all had turnover numbers $\geq$ 73 sec^{-1}. These activities are suitable for a commercial process where turnover numbers of about 5-10 sec^{-1} are the minimal requirement. We also constructed a synthetic butyrate operon that includes enzymes A-D in figure 1. We have shown that this operon produces enzymes A-D in an active soluble form in *E. coli*. All four enzymes have an activity >1.6 μmole/min/mg in crude extracts. This corresponds to a maximum theoretical rate of 16 g/L/h butyrate formation under industrial conditions (2-4 g/L/h is a good target). We introduced the synthetic butyrate operon into an *E. coli* strain that has all the native fermentation pathways eliminated by genetic deletion including *adhE*, *ldhA*, *pta-ack* and *frdBC* mutants. This quintuple mutant metabolizes glucose to acetyl-CoA and formate with the latter compound being converted to H_2 + CO_2. However, it is unable to grow anaerobically due to the inability to regenerate NAD^+ from NADH + H^+. In this strain, the synthetic butyrate operon was expected to restore growth by enabling the oxidation of NADH via the conversion of acetyl-CoA to butyryl-CoA. The quintuple mutant containing empty vector and vector plus the synthetic butyrate operon was grown on glucose anaerobically and the fermentation products were measured by HPLC. The strain containing the synthetic operon produced about 0.3 g/L butyrate and 0.008 g/L hexanoate. The identity of these products was confirmed by GC-MS. In contrast, the strain with the empty vector did not produce detectable amounts butyrate or hexanoate. During the production of butyrate and hexanoate about 1.8 g/L glucose was consumed. Thus, butyrate was produced and about 27% theoretical yield. Further work will focus on improving the yield and productivity butyrate and hexanoate.

We also engineered *E. coli* for the co-production of acetaldehyde and hydrogen. We cloned and purified acetaldehyde dehydrogenase from *Salmonella*. Its turnover number was 16 sec^{-1}. We then cloned and produced acetaldehyde dehydrogenase in wild-type *E. coli* and a strain that has the native fermentation

pathways eliminated by genetic deletion including *adhE*, *ldhA*, *pta-ackA* and *frdC*. When these strains are growing anaerobically, glucose is converted to pyruvate which is split to acetyl-CoA and formate with the latter compound being converted to H_2 + CO_2. Subsequently, acetyl-CoA was converted to acetaldehyde by the acetaldehyde dehydrogenase we introduced by genetic engineering. In the quintuple mutant growing on glucose anaerobically, about 50 μmole of acetaldehyde is produced from 120 μmole of glucose. However, under the conditions used formate accumulated and hydrogen was not produced. This strain also produced significant amounts of ethanol even though the AdhE enzyme was eliminated by genetic deletion. Further studies indicated that *E. coli* produces an alcohol dehydrogenase that uses acetaldehyde (rather than acetyl-CoA) as a substrate. An allyl alcohol selection was used to eliminate most of this Adh activity and acetaldehyde production was increased. We improved the conversion of formate to H_2 + CO_2 by dropping the pH of the growth medium to 6.0. Prior work by others had shown that the formate hydrogen lyase of *E. coli* is activated by lower pH values. Under optimal conditions, strain ZH136 converted glucose to acetaldehyde and hydrogen in a 1:1 ratio with a specific acetaldehyde production rate of 0.68 ± 0.20 g h^{-1} g^{-1} dry cell weight, and in 86% of theoretical yield. These results are encouraging that H_2 co-production will be a useful solution for elimination of unwanted electrons during the production of molecules more oxidized than glucose. Other CBiRC personnel are conducting feasibility studies on upgrading acetaldehyde to longer chain organic chemicals.

Other Relevant Work

To our knowledge, no other group has used a CoA-based pathway to produce hexanoic acid and bifunctional C6 compounds and *E .coli* has not been previously used for butyrate production. A number of other researchers have utilized heterologous expression of one or more of the enzymes leading from acetyl-CoA to butyryl-CoA for the production of 1-butanol or isopropanol in *E. coli* (2-6). Several additional papers describe the engineering of pathways to produce 3-hydroxybutyrate (7, 8). Our research parallels these previous studies as far as the production of butyryl-CoA, but we plan to extend the butyrate pathway to produce hexanoate and bifunctional C6 compounds. Nonetheless, prior studies on the production of C4 compounds via CoA derivatives provides information relevant to this project. Studies by Inui et al. (5) suggested that the lack of appropriate electron transfer proteins (EtfAB) impaired flux through from acetoacetyl-CoA to 1-butanol. EtfAB are needed for the activity of crotonyl-CoA reductase (CCR). We plan to use CCR from *Euglena* which uses NAD^+/NADH as a co-substrate rather than EtfAB. In other studies, Vadali et al. demonstrated that genetic modification *E. coli* (deletions, modifications) as well as manipulation of cofactor levels could be used to redirect acetyl-CoA into specific pathways to attain desired end-products (9, 10). Hanai, et al. successfully used the acetoacetyl-CoA pathway to produce isopropanol in *E. coli* in titers greater than that of native producers by using codon optimized synthesized genes from two *Clostridia* species and *Thermanaerobacter brockii* (4). Others expanded this line of research with a modified strain for isopropanol synthesis that produced 227 mM isopropanol, and a sixth the amount of acetate compared to wild type thus demonstrating the ability to significantly target synthesis of a desired molecule with a concomitant shift of flux away from a competing allosteric regulator (6). Close collaboration with investigators in Thrust 2 to define metabolic fluxes involved in the production of targeted CoA intermediates will be necessary. In general, the scientific literature has shown *E. coli* metabolism to be highly malleable in regards to redox state and flux manipulations making it amenable to the introduction of heterologous pathways for green chemical production (5, 6, 11, 12). Hence, our goal to express non-native enzymes in *E. coli* to produce 4- and 6-carbon CoA intermediates from glucose for downstream modifications by Thrust 3 has a solid basis in the literature.

Plans for the Next Year

We will work on increasing the yield and productivity of short chain carboxylic acids by fermentation. The synthetic operon which we expressed in *E. coli* (produces enzymes A-D in figure 1) did not include enzymes for removal of CoA from butyric acid. We presumed this would occur via endogenous enzyme systems and this was observed at least to some extent. However, the endogenous enzymes for CoA removal could be

limiting the rate of butyrate production. We will engineer CoA removal systems into *E. coli* including especially thioesterases.

We will also work on producing the specificity of product production. Currently we are producing mixtures of butyric, hexanoic and octanoic acids. We will try to produce just one compound in higher yields. To accomplish this we will work to define the endogenous systems the mediate the conversion of CoA derivatives to their corresponding carboxylic acids + CoA and replace them with alternative more specific systems to be identified by a variety of methods described above. Subsequently, cells will be grown on glucose and fermentation products measured by HPLC and yield and productivity determined.

We will continue work on the production of odd-chain length carboxylic acids by engineering systems that produce propionyl-CoA into *E. coli*. Propionyl-CoA is expected to serve as a substrate for enzyme A figure 1 such that this reaction produced 3-hydroxyvaleric acid. Subsequently, the 3-hydroxyvaleric acid will then move through enzymes B-D yeilding valeric acid after CoA removal. This same approach is expected to work for the production of longer odd chain length carboxylic acids. Controlling product specificity will require appropriate CoA removal enzyme as described above.

Expected Milestones and Deliverables

We expect to produce the following compounds.

1. heptanoic acid
2. 3-hydroxyheptanoic acid

Member Company Benefits

The proposed task will provide microbial catalysts for the production industrial chemicals or platform chemicals.

Commercialization / Technology Transfer

This will be done by consulting with member companies. There has been no technology transfer to date.

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NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.3 – Acetyl-CoA/Propionyl-CoA Synthetase:** Biocatalysts for Diversifying Precursor Pools for FAS/PKS Synthesis

Thrust: Thrust 1 - New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
David J. Oliver, Basil J. Nikolau	**02/17/2012**	**3/1/2011 to 2/29/2012**

ERC Team Members

Project Leader (until **August, 2011)***:*** David Oliver, Iowa State University
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Statement of Project Goals

The specific goal of this project is to develop biocatalysts (enzymes and enzyme systems) that can provide novel acyl-CoA precursors for the fatty acid synthase (FAS)/polyketide synthesis (PKS) system. In most biological systems, products generated by FAS/PKS are straight chain and of chain lengths of even number carbon atoms (e.g. 8,10, 12, etc.). This is the result of the fact that acetate is used to both prime and extend the acyl chain. The primers for these FAS/PKS systems are introduced into the biosynthetic machinery as acyl-CoAs. In order to diversify the range of products that can be generated by FAS/PKS systems, this project seeks to discover and/or bioengineer acyl-CoA synthetases that can produce acyl-CoA primers that are odd numbered (i.e., propionyl-CoA) or branched chain (i.e., isobutyryl-CoA, isovaleryl-CoA or 2-methylbutyryl-CoA) primers. The incorporation of such primers into the FAS/PKS biosynthetic machinery would diversify the products of FAS/PKS by enabling the biosynthesis of odd-numbered straight-chain carboxylic acids, iso-branched odd and even numbered carboxylic acids, or anteiso-branched odd and even numbered carboxylic acids. The immediate goal of the project is to provide well characterized, high activity enzymes that can generate acetyl-CoA, propionyl-CoA, isobutyryl-CoA, isovaleryl-CoA or 2-methylvaleryl-CoA for Thrust II.

Project's Role in Center's Strategic Plan

The objective of CBiRC is to create new biologically-derived platform chemicals to replace existing petroleum-derived chemicals for the synthesis of commodity chemicals. This will be accomplished by creating a new series of biological precursors modified from intermediates of polyketide/fatty acid synthesis that can then be converted by chemical processes into feedstock compounds. The biochemical catalysts that will be created by Thrust 1 will be designed to mimic existing PKS/FAS systems but altered to create and release a variety of small reaction intermediates instead of the long chain fatty acids that the systems currently produce. In order to accomplish this goal we will need to identify or engineer enzymes that can create novel acyl-CoA molecules that

can serve as precursors for the systems, modified ketoacyl-synthases that can use these novel substrates and new thioesterases and methylketone synthases that can release the desired intermediates. The purpose of this project is to develop the acyl-CoA synthetases that can provide novel acyl-CoA primer molecules as substrates for the process.

This project will begin by developing a modified acetyl-CoA synthetase that can be used for several purposes. It will allow us to develop enzymology capabilities needed to work with this family of proteins. It will also provide a reagent for Thrust II that will allow them to modify *E. coli* to increase its capacity to use acetate as a substrate. This will be an important organism for the Center's long-term vision in that it will allow for the experimental modification of the rate of acetyl-CoA production and thus evaluate the effect of altering metabolite flux in the middle of the pathway. Our longer term goals are to develop acyl-CoA synthetases that will provide propionyl-CoA and branched chain CoAs as precursors.

Fundamental Barriers and Methodologies

In order for this project to be successful two sets of initial goals need to be accomplished. First, three different acyl-CoA synthetases, specific for acetate, propionate, and isobutyrate need to be discovered (or bioengineered) and characterized. These would provide the key biocatalysts for the project. Second, once these enzymes are available, they will need to be modified to provide maximum activity when expressed in *E. coli* or yeast. Studies in this and other laboratories have shown that these enzymes are controlled by two independent post-translational systems, an oxidation of enzyme thiol groups and an acylation of the enzyme active site. We will need to understand the biochemistry of these mechanisms and to create mutants that are not regulated in order to achieve maximum expression.

Achievements

(Prior to 2/28/2011):

Isolation, expression, and characterization of acetyl-CoA synthetase, propionyl-CoA synthetase, and isobutyryl-CoA synthetase.

Table 1. Substrate specificities for three acyl-CoA synthetases.

Enzyme	Substrate	Km (mM)	Vmax (nmole mg^{-1} min^{-1})
PCS (*E. coli*)	acetate	2.1	1.7
	propionate	0.05	4.2
	isobutyrate	nd	nd
ACS (*Arabidopsis*)	acetate	0.04	48.7
	propionate	0.25	2.3
	isobutyrate	nd	nd
ICS* (Pseudomonas)	acetate	10.6	1.78
P. chlororaphis	propionate	1.10	12.4
	isobutyrate	0.14	10.7
nd = no detectable activity			

Genes for three acyl-CoA synthetases have been isolated and the respective proteins expressed in *E. coli* and characterized *in vitro*. The preferred substrates for propionyl-CoA synthetase (PCS), Arabidopsis acetyl-CoA synthetase (ACS) and the *Pseudomonas chlororaphis* isobutyryl-CoA synthetase (ICS) were shown to be propionate, acetate and isobutyrate, respectively (Table 1).

Overcoming post-translational regulatory systems in acyl-CoA synthetases.

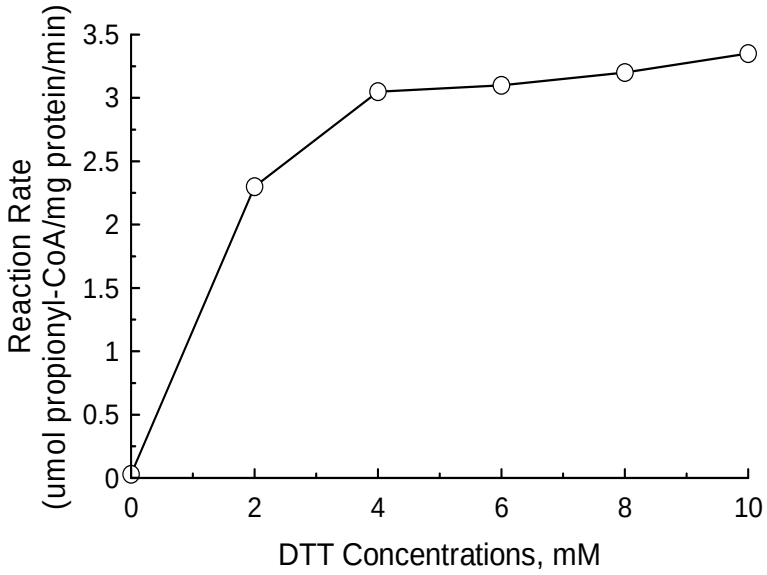

Figure 1. Activation of *E. coli* propionyl-CoA synthetase by DTT.

The *E. coli* PCS showed low activity *in vitro*. The activity was increased 5- to 10-fold after incubation with the thiol reducing agent, dithiothreitol (DTT) (Figure 1). This activation was reversed by treatment with the thiol oxidizing agent, diamide. This type of reversible activation and inactivation by reduction and oxidation normally signifies the involvement of a pair of cysteine residues that are critical to catalysis; namely their chemical reduction results in the activation of the enzyme and their chemical oxidation causes inactivation.

The *E. coli* PCS contains eight cysteine residues. Modelling of the *E. coli* PCS sequence on the known Salmonella PCS structure did not identify any obvious candidate Cys residues that could form intramolecular disulfide bonds. However, gel electrophoresis experiments indicated that the enzyme has the same apparent molecular weight in the presence and absence of DTT, which suggests that the reactive regulatory disulfide bond is intramolecular. To identify the cysteine residues that were members of this disulfide bond pair, eight mutant forms of PCS were created where each one of the individual cysteine residues was changed to alanine. The Vmax for these mutant enzymes with and without DTT activiation were then determined (Table 2). Mutation of a cysteine involved in the active site would be expected to strongly impact the Vmax. Mutation of a cysteine involved in the DTT-reactive disulfide bond would be expected to produce an enzyme that was fully active in the absence of DTT. All of the mutant enzymes had a Vmax that was

approximately equivalent to the wildtype enzyme. For six of the eight mutants, the activation by DTT was equivalent to that seen with the wildtype proteins. For two mutants however, C128A and C315A, the mutant PCS was fully active without DTT treatment. This indicates that the regulatory disulfide bond occurs between Cys128 and Cys315. These results also demonstrate that enzymes with either cysteine mutated to alanine are fully active in the absense of DTT.

Table 2. Site-directed mutagenesis of *E. coli* PCS to identify cysteine residues involved in reversible redox regulation.

Enzyme	Vmax (nmol propionyl-CoA mg protein^{-1} min^{-1})	DTT Activation (fold)
PCS – WT	**9.98**	**5.0**
C47A	**10.02**	**4.0**
C236A	**11.28**	**10.3**
C279A	**4.98**	**4.5**
C431A	**16.13**	**4.2**
C450A	**15.62**	**7.8**
C604A	**8.65**	**10.2**
C315A	**18.33**	**1.5**
C128A	**15.24**	**1.5**

These Cys-to-Ala mutants do not produce a constitutively active enzyme because a second regulatory mechanism occurs. In the case of PCS it is a propionylation of the active site lysine with propionyl-CoA acting as the propionate donor (Figure 2). This inhibition is reversed by the enzyme, CobB.

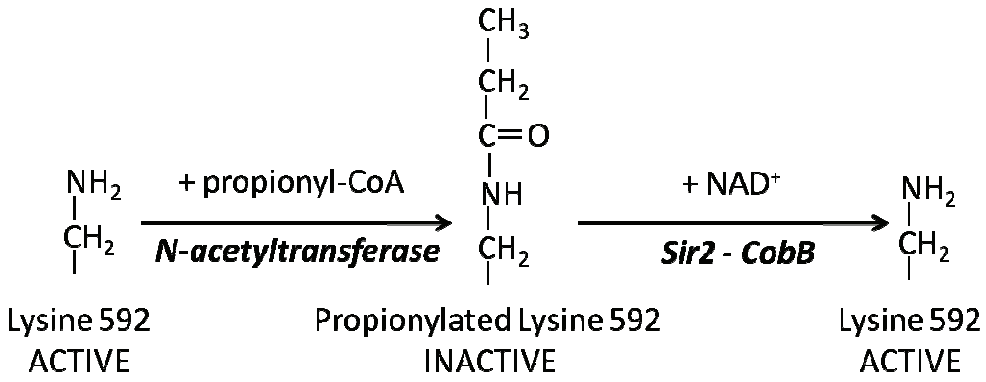

Figure 2. Regulation of PCS by reversible propionylation.

The regulation by propionylation and disulfide oxidation and reduction are independent events. Both DTT and CobB treatment increased PCS activity independently ~5- fold but the two treatments together increased PCS activity by 25-fold (Figure 3).

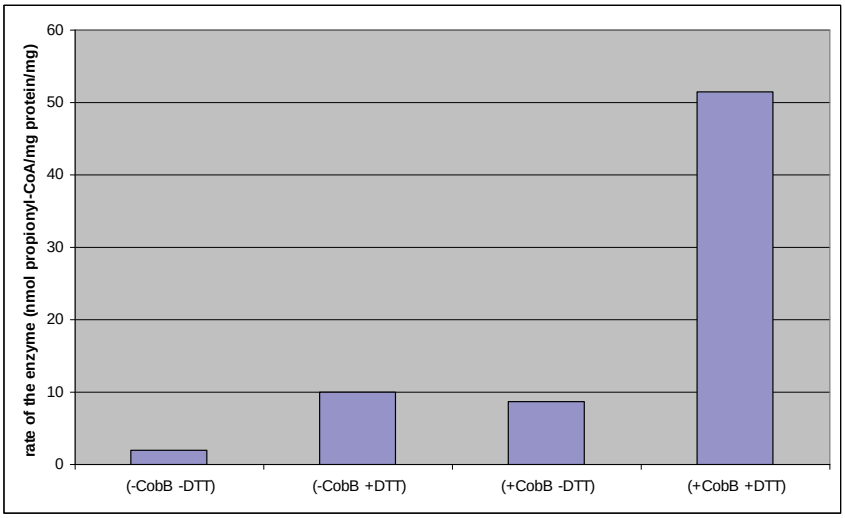

Figure 3. Regulation by DTT and CobB are multiplicative, indicating that they occur by independent mechanisms.

At present, we have identified two strategies to overcome the regulation by acylation. In some cases it may be possible to express a PCS protein from a different phylogenetic source that is resistant to propionylation and thus bypasses the regulation. For example, the Arabidopsis enzyme is not regulated by acylation in *E. coli*. We have also developed a mass spectroscopy-based method that may be useful for identifying mutations that prevent propionylation when PCS is expressed in *E. coli*.

Achievements from 3/1/2011 to 2/29/2012:

Modification of acetyl-CoA synthetase substrate specificity.

Recently, we tested whether the substrate specificity of Arabidopsis ACS (i.e. acetate) can be changed to the substrate specificity observed for *Psuedomonas chlororaphis* ICS (i.e. isobutyrate). Using homology modeling of Arabidopsis ACS and *Psuedomonas chlororaphis* ICS, four active site residues in ACS were mutated to the corresponding residues in ICS. Single, double and quadruple mutant constructs were expressed in *E. coli* and purified. Activity with specific substrates was determined by using an isotope labeled ^{13}C-dependent assay (Table 3). These experiments, indicate that the Val^{388}Ala mutation reduces synthetase activity with acetate and increases activity on propionate and isobutyrate. The Trp^{416}Gly mutation decreases synthetase activity with acetate and to a lesser extent, propionate and isobutyrate. In a Val^{388}Ala; Trp^{416}Gly double mutant, enzyme activity on propionate and isobutyrate are similar to that of wild-type synthetase activity on acetate. When four active site mutations are present, the substrate specificity dramatically shifts to isobutyrate at a rate >10-fold higher than the rate at which the wildtype enzyme acts on acetate. We have recently developed a colorimetric assay that will be used to confirm these initial findings.

Table 3: *In-vitro* substrate-specific enzyme activity of Arabidopsis acetyl-CoA synthetases containing active-site mutations

Enzyme	Rate with different substrates (µmole/min/mg protein)		
	Acetate	Propionate	Isobutyrate
Wild-type	3.30	0.43	0.07
$Trp^{416}Gly$	0.02	0.14	0.01
$Val^{388}Ala$	0.11	1.30	1.21
$Ile^{312}Ala$, $Thr^{313}Tyr$	0.12	0.11	0.66
$Val^{388}Ala$, $Trp^{416}Gly$	0.58	3.5	4.16
$Ile^{312}Ala$, $Thr^{313}Tyr$, $Val^{388}Ala$, $Trp^{416}Gly$	0.88	0	54.96

Other Relevant Work

A manuscript describing the dithiol regulation of acyl-CoA synthetases has been accepted for publication. While other groups have worked on regulation by acylation, no one has published a mechanism for overcoming this feedback inhibition.

Plans for the Next Year

- Confirm Arabidopsis ACS mutant activities on different substrates using a colorimetric assay.
- Compare activities of mutant ACS enzymes to those of *E. coli* ACS, *E. coli* PCS, and *P. chlororaphis* ICS using colorimetric assay.
- Generate and characterize $Ile^{312}Ala$ and $Thr^{313}Tyr$ single ACS mutants to determine their individual effects on substrate specificity.
- Study the *in vivo* effects on acyl-CoA pools in *E. coli* expressing ACS, ACS mutants, PCS and ICS enzymes via LC-MS-MS analysis.
- Analyze fatty acid profiles from *E. coli* expressing ACS, ACS mutants, PCS and ICS enzymes via GC-MS analysis. This will access the impact of new FAS primers on fatty acid synthesis products.

Expected Milestones and Deliverables

The deliveries for this project are the genes for the modified forms of these enzymes along with the knowledge of how to manipulate and assay them. We expect these to be done within the next two years and delivered to Thrust II.

Member Company Benefits

A disclosure has already been released on the redox regulation mechanism and one of the partner companies has expressed interest in this technology. An early draft of the manuscript describing this biocatalyst has been forwarded to them. We will keep them informed as additional biocatalysts become available and as we gain information on the potential of these enzymes to modify metabolism in *E. coli*.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.4 – Acyl-CoA Carboxylases:** Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Basil J. Nikolau	**2/17/12**	**03/01/2011 to 02/29/2012**

ERC Team Members

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Other Faculty: Peter J. Reilly, Iowa State University
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Other Personnel: Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

The goal of this project is to develop acyl-CoA carboxylases (ACCase) that can activate diverse acyl-CoA molecules to produce novel substrates for 3-ketoacyl-ACP synthases or other KS components of polyketide synthases (e.g., pyrone synthase). Normally, acetate units are activated for polyketide synthesis by carboxylating acetyl-CoA to malonyl-CoA. The loss of the CO_2 group in subsequent reactions drives the condensation reaction catalyzed by the 3-ketoacyl synthases. One of the other projects in this thrust is designed to produce branched-chained, odd-numbered acyl-ACP molecules and pyrones. In order to achieve these goals, this project seeks to enhance and modify the malonyl-CoA substrate that will be used in the biosynthesis of these products; i.e., we will need to develop acyl-CoA carboxylases with enhanced activity and altered substrate specificities.

Project's Role in Center's Strategic Plan

The goal of the thrust is to identify or bioengineer biocatalysts that can be used to produce a diverse group of biochemicals from the intermediates of fatty acid biosynthesis. While the products of this pathway are normally even-numbered and straight-chained, our intention is to also produce molecules that contain chemical functionalities at the omega-end of the product molecules (e.g., branched-chain, odd-numbered carbon-compounds, ring-structures, hydroxylated or halogenated products). In order to accomplish this, we will need to incorporate altered primers into the polyketide synthesis biocatalysts and enhance the supply of malonyl-CoA, the extender substrate of polyketide synthesis biocatalysts. This project is designed to produce biocatalysts that address the latter question, enhancing the supply of malonyl-CoA. This will be accomplished by creating modified acetyl-CoA carboxylases as specific biocatalysts. Initially, we will survey the acyl-CoA carboxylases in a range of microbial systems in order to identify enzymes with diverse substrate specificities. Structural analyses and site-directed mutagenesis will be used to extend the natural range of substrates used and to create the necessary biocatalysts for the project that will use unusual acyl-CoAs as substrates. The Reilly group will construct a database of the acyl-CoA carboxylase

genes, proteins, and structures in the literature and public databases.

Fundamental Barriers and Methodologies

- Purification of the heteromeric ACCase from many sources is difficult due to the subunits dissociating during purification. Because there are multiple genes encoding each of the ACCase subunits [biotin carboxyl-carrier protein (BCCP), biotin carboxylase (BC), carboxyltransferase alpha (CT-alpha) and beta (CT-beta) subunits], which is a large multimeric complex, subsequent purification of ACCase result in a heterogeneous mixture of isoforms within holo-ACCase. To overcome this barrier, we have built a heterologous expression system in *E. coli* to express each ACCase subunit individually and in combination with the other subunits of ACCase.
- Previous studies indicate that over-expression of ACCase subunits in *E. coli* results in the accumulation of predominantly insoluble, inactive biocatalysts. By co-expressing subunits we have increased the solubility and functionality of the expressed ACCase.

Achievements

Before 8/31/09:

- Created recombinant plasmids for the co-expression in *E. coli* of the components of the plant-derived (*Arabidopsis*) heteromeric ACCase. This enzyme is composed of a combination of four distinct subunits: BCCP, BC, CT-alpha, and CT-beta. Further diversity is possible as there are two versions of the BCCP component (BCCP1 and BCCP2). Expression constructs that can co-express different combinations have been built. These combinations are:
    1. BC and BCCP1
    2. BC and BCCP2
    3. CT-alpha and CT-beta
    4. BCCP1, BC, CT-alpha and CT-beta
    5. BCCP2, BC, CT-alpha and CT-beta
- Co-expression for each of the above combinations was achieved.
- Collected all amino acid sequences deposited at NCBI and three-dimensional structures for carbon-carbon bond-forming ligases from EC 6.4.1.1 to EC 6.4.1.7 (no amino acid sequences for EC6.4.1.6).
- Found that all of these enzymes share similar BC and BCCP domains, but they differ in whether all domains are linked in one polypeptide chain, or differ in the order of the domains on a single polypeptide, and in the type and number of CT domains that constitute each enzyme system.

From 9/1/09 to 2/28/10:

- Upon further consideration, two new co-expression systems were generated to study the co-expression of BCCP1 and BCCP2 isoforms in concert with CT-alpha and CT-beta. This could lead to a better understanding of the interactions of the acetyl-CoA specific subunits (CT-alpha, CT-beta) with the biotinylated BCCP. These combinations are:
    6. BCCP1, CT-alpha and CT-beta
    7. BCCP2, CT-alpha and CT-beta
- Soluble expression of BC/BCCP1 and BC/BCCP2 constructs has been optimized and fractions

have been extracted from *E. coli*. These soluble fractions have been studied via FPLC and non-denaturing-PAGE analysis. Both experiments indicate that these heterologously expressed proteins are interacting as potentially large oligomers (~900,000 Daltons).

From 3/1/10 to 2/28/11:

- BC, BCCP1, and BCCP2 were each over-expressed in *E. coli* BL21 (DE3), C41 (DE3), and C41(DE3) cells, respectively and yield was optimized to 2-4 mg/ml of pure protein. Purification of BC, BCCP1, and BCCP2 was optimized to greater than >90%, >96%, and >96% purity, respectively. Individual subunits were subjected to electrophoresis on non-denaturing polyacrylamide gels (Native-PAGE) and populations representing monomer, dimer, tetramer, and large oligomeric forms were observed (Figure 1).

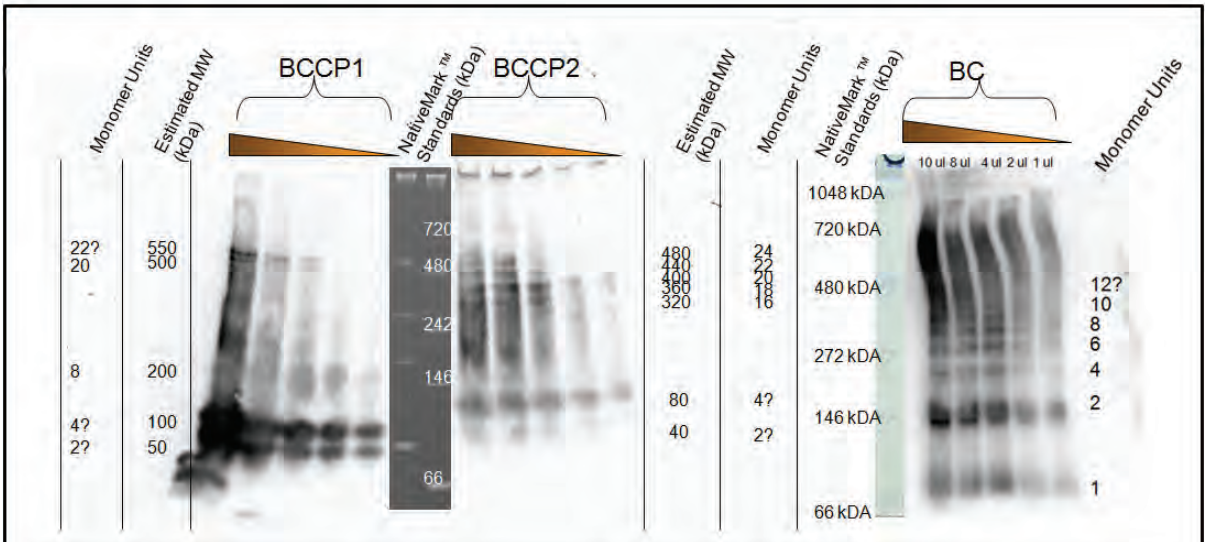

Figure 1: Native-PAGE analysis of BCCP1, BCCP2, and BC. Gels were 4-20% acrylamide (Biorad®). BC was run separate from BCCP1 and BCCP2. Estimated molecular weights (kDa) and potential monomer units are indicated.

- The same protein preparations were subjected to size-exclusion FPLC chromatography, which was consistent with Native-PAGE analysis. To test the importance of hydrophobic and/or ionic effects on protein oligomers, all three preparations were treated with different concentrations of sodium chloride (50, 100, 200, 400 and 800 mM), and analyzed via FPLC gel filtration chromatography. BCCP1 (Figure 2) and BCCP2 prefer the higher oligomeric state at high salt concentrations, suggesting hydrophobic effects influence their interaction. BC showed little change over the entire range of salt concentrations tested.
- Acyl-CoA Carboxylase domains were classified by their amino acid sequence similarity and three-dimensional structure similarity. Each domain of ACC (BC, BCCP, CT-alpha and CT-beta) was treated separately. Each domain has only one family, demonstrating that all sequences are similar for one particular domain. All data are available on ThYme database (www.enzyme.cbirc.iastate.edu).
- Initial work had been done on the enzyme kinetics of BC/free biotin, BC/BCCP1, and BC/BCCP2.

From 3/1/2011 to 2/29/2012:

- Due to the large family sizes for the BC (5396 sequences) and BCCP (5305 sequences) components, phylogenetic trees for BC and BCCP were constructed using a representative subset of sequences for each family. BC has eight subfamilies and three outlier sequences. BCCP has four subfamilies.
- CT-alpha and CT-beta were co-expressed in a pETDuet multiple cloning site vector and subsequently co-purified to test for protein-protein interactions between CTalpha and the two isoforms of CT-beta; the CT-beta isoform is generated from a naturally occurring C784U posttranscriptional modification that causes a S265L amino acid substitution. Only the CT-beta isoforms contain a 6X-his-tag. Therefore, any CT-alpha present in fractions purified over a nickel affinity column must result from physical interaction with CT-beta. It appears that the presence of leucine at position 265 improves CT-beta affinity for CT-alpha, as evident by the presence of CT-alpha in the flow-through (star in Figure 2). In addition, overall protein titer and solubility is also improved in the L265 isoform compared to S265. Further kinetic testing on each CT complex is ongoing.
- Native gel analysis also shows that leucine at position 265 creates two new co-populations of CT complexes (Figure 3)

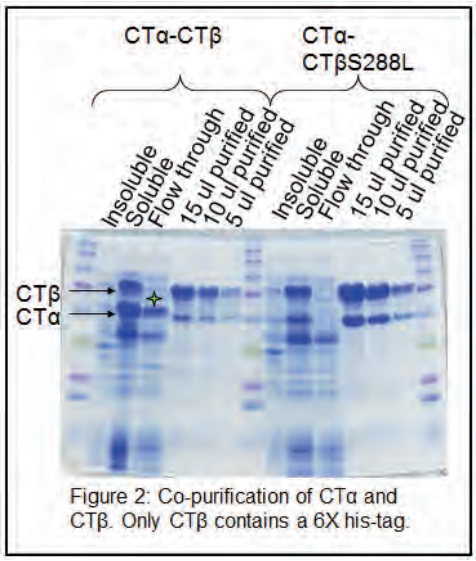

Figure 2: Co-purification of CTα and CTβ. Only CTβ contains a 6X his-tag.

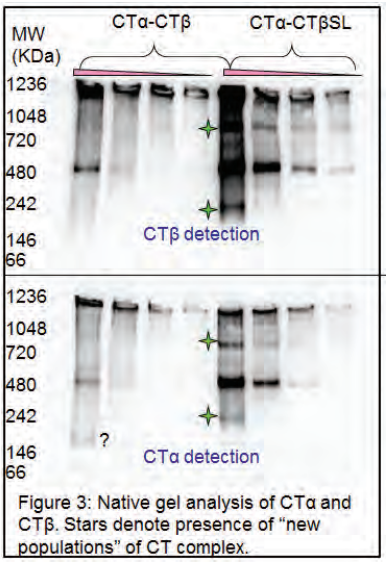

Figure 3: Native gel analysis of CTα and CTβ. Stars denote presence of "new populations" of CT complex.

- Performed circular dichroism (CD) experiments on purified BCCP1 and BCCP2 (Figure 4). Jfit program was used to determine % alpha-helix and beta-strand from the CD data for each BCCP isoform. These data are in close agreement with secondary structure prediction using CHOFAS (Table 1). Together, these data suggest that both BCCP isoforms fold in a similar manner and that specific residues, likely within the more variable N-terminus, may be responsible for the differences observed in protein-protein interactions.

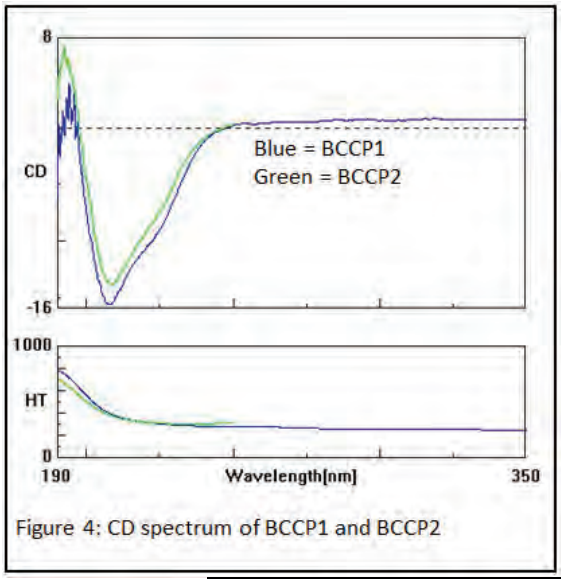

Figure 4: CD spectrum of BCCP1 and BCCP2

	CD Data			CHOFAS Data		
	alpha helix	beta strand	Random coil	alpha helix	beta strand	Random coil
BCCP1	11.7	39	49.3	21.5	46.2	32.3
BCCP2	19.1	33.7	47.3	12.4	50	37.6

Table 1: Percent alpha helix, beta strand, and random coil for BCCP isoforms determined by CD spectra and CHOFAS secondary structure prediction algorithm.

- Optimized expression of BCCP1 and BCCP2 in minimal media for use in solution NMR studies.
- Began further optimization of BC purification for X-ray crystallography.

Other Relevant Work

In addition to acetyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase (MCCase) is being studied. This enzyme catalyzes the carboxylation of 2-methylcrotonyl-CoA to form 3-methylglutaconyl-CoA and contains only two subunits, an MCCA which contains both BC and BCCP domains, and MCCB, which contains a functional CT domain. In *Arabidopsis thaliana* MCCA exists as both a long and a short isoform, generated by alternative splicing of *mcca* mRNA. Computational modeling of both MCCA isoforms suggests that both may fold into potentially active enzymes, with the difference between the two being an alpha-helix distal to the active site. It is unclear, however, whether the presence or lack of this helix has an effect on protein-protein interactions, which could affect activity. By understanding the diversity within and between ACCase and MCCase, it may be possible to understand the biochemical control of substrate specificity at the CT active site.

Plans for the Next Year

- Completion of kinetic analysis for ACCase (turnover number, substrate Km/Vmax, etc.)
- Define the oligomeric status for all subunits of ACCase and MCCase for each subunit

individually and in concert with combinations of subunits.

- Solution NMR (HSQC and NOESY) studies of BCCP1 and BCCP2 to solve solution structures for each isoform.
- Initial X-ray crystallography studies of BC to compare Arabidopsis BC structure to that of known bacterial structures.
- Determine dissociation constants for each subunit of ACCase and MCCase.
- Construction and statistical analysis of phylogenetic trees for the remaining families of BC, CT-beta and selection of genes(s) of interest to synthesize.
-

Expected Milestones and Deliverables

- Purification and characterization of ACCase complexes by May, 2012.
- Purification and characterization of MCCase complexes by December, 2012.
- Phylogenetic analysis for all acyl-CoA carboxylase families, by October, 2011.
- Based on phylogenetic analyses of ACCase subunits from diverse organisms, synthesis and expression of potentially novel acyl-CoA carboxylase enzymes, by October, 2012.

Member Company Benefits

This project will generate novel biocatalysts that can generate "elongating substrates" for fatty acid synthases and/or polyketide synthases. Depending on the novel substrate that will be generated, the use of these biocatalysts will result in the incorporation of internal methyl- or ethyl-branches in the resulting alkyl chain. Another potential benefit of this project derives from the fact that the acyl-CoA carboxylase biocatalyst is considered to be an important regulatory reaction of fatty acid synthesis; thus, this research has potential to enhance the production of fatty acids. These are questions that many of CBiRC's industrial partners would like to address, in order to enhance their biorenewable chemical platforms based upon fatty acid biosynthesis.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.5 – Methylketone Synthase/Thioesterase:** Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for Production of Short-Chain Methylketones.

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Eran Pichersky	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leaders: Dr. Eran Pichersky, Dr. Thuong Nguyen, Mr. Geng Yu – University of Michigan
Dr. Joseph Noel, Dr. Yongxia Gao – Salk Institute

Statement of Project Goals

Tomato methylketone synthase1 (MKS1) and MKS2 convert intermediates in the fatty acid biosynthesis pathway, namely 3-ketoacyl-ACP, to mathylktones. In tomato, MKS2 "grabs" the C12, C14 and C16 3-ketoacyl-ACPs and hydrolzyes the ACP. MKS1 then decaroxylates the resulting products to give C11, C13 and C15 methylketones. The overall aim of this project is to use these two enzymes to terminate fatty acids at an earlier cycle in the chain elongation process, to provide methylketones of shorter chain length such as C5 and C7, and to achieve a high level of production of such methylketones. To achieve the synthesis of short chain methylketones, we will use two approaches: Engineering the existing tomato MKS1 and MKS2 by in vitro mutagenesis based on information derived from the crystal structure of the protein (this work will be done with Dr. Noel's lab at the Salk) and by looking for additional natural variants of MKS1 and 2 (which will subsequently be structurally characterized by Noel's group).

Project's Role in Center's Strategic Plan

Providing plant genes for enzymes that produce short methylketones.

Fundamental Barriers and Methodologies

Cloning genes from plants, expression in *E. coli*, testing activities in vitro using in-house synthesized substrates.

Achievements

Goal 1: Develop an MKS2 that catalyzes the formation of short-chain methylketones.

Task 1: Perform *in vitro* mutagenesis of tomato MKS2.

Progress: The lack of structural information on MKS2 is slowing down our work on in vitro mutagenesis. We have obtained several mutants so far based on some structural predictions (from comparisons with other related proteins), but so far we have not obtained mutants that

better hydrolyze shorter precursors.

Task 2: Clone and characterize new MKS2 natural variants from various tomato accessions and from other species, express them in *E. coli* and characterize their activity

MKS2 proteins are widely distributed in the plant kingdom and they can produce methylketones when expressed in E. coli. We had previously analyzed MKS2 from two tomato species and from Arabidopsis (Arabidopsis has three functional MKS2 genes). We expressed these genes with varying success in E. coli and showed their activity by measuring methylketone production (by GC-MS). We have observed the production of methylketones from the MKS2 cDNAs of the two different tomato species and from two of the three Arabidopsis MKS2 cDNAs. Promisingly, each MKS2 produces a somewhat different range of methylketones. One tomato MKS2 and one Arabidopsis MKS2 produce predominantly C7 and C9 methylketones, and less of the longer ones. We also identified MKS2 from additional plant species, including momocots (rice, corn) and a gymnosperm(Sitka spruce), and they too produce methylketones when expressed in E. coli.The two tomato MKS2 cDNAs have been sent to Thrust 2 group members for optimization for microbial expression. We also purified these proteins and did in vitro enzymatic assays. This year we have concentrated on the plant rue (Ruta graveolens), which is known to produce mostly 2-nonanone. We have obtained several cDNAs encoding MKS2 from this plant, and are in the process of testing the encoded proteins for activity.

Task 3: Work with Noel's group to structurally characterize MKS2 enzymes.

Progress: All the MKS2 cDNAs described above have been sent to Dr. Noel's lab and they have begun the structural work. In addition, we are working with the Noel's group to develop MKS1-MKS2 fusion proteins that will have higher levels of activity and will also be more amenable to structural investigations. Thus far we have obtained atomic resolution structures of several MKS1 orthologs from cultivated tomato (S. lycopersicum – see below), verified that they catalyze decarboxylation of beta-ketoacids and have identified by protein x-ray crystallography, divalent metal-binding to one member of this SlMKS1 family that contribute to decarboxylation.

Goal 2: Improve the decarboxylase activity of MKS1 with shorter 3-ketoacids

Task 1: Perform *in vitro* mutagenesis of tomato MKS1.

Progress: Together with the Noel group, we have now obtained several MKS1 mutant proteins which are much more active with shorter 3-ketoacids (e.g., C7) than with C14 and have verified and extended the structural analysis of these mutants at near atomic resolution.

Task 2: Clone and characterize new MKS1 natural variants from various tomato accessions, express them in *E. coli* and characterize their activity

Progress: Plants outside the tomato genus Solanum do not have proteins that are closely related to MKS1. However, the cultivated tomato (S. lycopersicum) has at least three MKS1 homologs, and we are now studying then in some detail. Some have already been observed to be more active with short 3-ketoacids, but we need to further characterize them.

Task 3: Work with thrust 2 to analyze flux in *E. coli*

Progress: The tomato MKS1 cDNA, two tomato MKS2 cDNAs and the Arabidopsis MKS2 cDNAs have already been send to Thrust 2 investigators and some have already been expressed, with the results that methylketones have been produced.

Other Relevant Work

Plans for the Next Year

Analysis of rue MKS2 for substrate and product specificity and 3D analysis.

Expected Milestones and Deliverables

Several enzymes that can synthesize a range of short-chain methylketones.

Member Company Benefits

A patent application for the plant enzyme Methylketone Synthase 2, a thioesterase that hydrolyzes 2-ketoacyls, has been filed. This enzyme will be very valuable for producing short methylketones in bacteria and plants. A two-year license was obtained by Monsanto in 2011 for evaluation of the potential of this gene in plant defense.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T1.6 – Thioesterases:** Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date (in U.S. date format):	Reporting Period:
Basil J. Nikolau	**02/17/12**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Basil J. Nikolau, Iowa State University
Other Faculty: Peter J. Reilly, Iowa State University
Graduate Students: David Cantu, Yingfei Chen, and Fuyuan Jing, Iowa State University
Undergraduates: Jarmila Tvaruzkova, Iowa State University (Charles University, Prague)
Other Personnel: Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

The goal of this project is to identify and characterize novel biocatalysts from plant and microbial polyketide synthase (PKS) systems for the purpose of diversifying the fatty acid synthase (FAS) systems of *E. coli* and the yeast *Saccharomyces cerevisiae*. This project specifically targets enzymes that could be used to prematurely terminate FAS at shorter chain lengths than normal. Specifically, we have targeted acyl-ACP thioesterases (EC 3.1.2.14 and EC 3.1.2.21) as the biocatalysts that will prematurely terminate FAS, and acyl-CoA thioesterases (EC 3.1.2.2 and EC 3.1.2.20) as the biocatalysts that can terminate CoA-dependent acyl ester biosynthetic pathways. Initial goals aim to clone and express orthologs of these biocatalysts from diverse biological sources that are known to show distinct substrate specificities. These proteins will be characterized in order to determine the structure-function relationship to construct biocatalysts with increased catalytic efficiency and altered substrate specificity for shorter fatty acids. In parallel, we will construct databases of all the thioesterase genes, proteins, and structures uncovered in the literature and public database sources.

Project's Role in Center's Strategic Plan

One of the major goals of the Center is to create a biological system based on FAS/PKS, which can produce a suite of chemicals that are shorter than 6- or 8-carbon atoms. One means for achieving this goal is to find biocatalyst(s) for stopping the elongation process of FAS at less than 8-carbon atoms. Normally, FAS in *E. coli* and yeast is terminated at 16 and 18 carbon atoms. However, plant systems exist that can terminate the elongation process of FAS with different versions of acyl-ACP thioesterases that have specificity for chain lengths of 8, 10, 12, and 14 carbon atoms. The goal of this project is two-fold: 1) Find and characterize the molecular details of the nature of these thioesterases that display different substrate specificities; and 2) based on the understanding of the design-principle of these thioesterases, create by mutagenesis thioesterases that have the desired substrate specificities.

Fundamental Barriers and Methodologies

To design novel biocatalysts that can prematurely terminate FAS at shorter chain lengths, the fundamental knowledge that is required is how to elucidate the structure-function relationship of this biocatalyst. However, it's not well understood how acyl-ACP thioesterases recognize different substrates due to the lack of structures. To overcome this barrier we will determine the structures of acyl-ACP thioesterases that have different substrate specificities. Briefly, several acyl-ACP thioesterases will be over-expressed, purified and used to study the structure-function relationship. Novel thioesterases can then be rationally designed based on this knowledge. A high-throughput method will also be developed to test the bioactivity of different thioesterases and their mutants.

Achievements

Prior to 2/28/2011:

- Optimized the recombinant expression in *E. coli* of a FatB-type thioesterase (TE) isolated from cDNAs of oil palm seeds (*Elaeis guineensis*) - EgPTE; a tissue that normally produces large quantities of 12-carbon fatty acids.
- Optimized the purification of recombinant EgPTE, with yields in the range of 10-20 mg protein per liter of *E. coli* culture.
- Isolated full-length cDNA clones for three new FatB-type thioesterases from developing coconuts, a tissue that is known to accumulate 8-carbon fatty acids and small quantities of 6-carbon fatty acids.
- Three new acyl-ACP TEs were isolated from developing seeds of *Cuphea viscosissima*, a plant that accumulates 14% octanoic acid and 72% decanoic acid within seeds.
- Thioesterase sequences obtained from public databases were classified into 25 families based on sequence and three-dimensional structure similarity, and all TE sequences are collected in the constantly-updated ThYme database (www.enzyme.cbirc.iastate.edu).
- All plant and bacterial acyl-ACP TEs involved in type II fatty acid biosynthesis were classified as family TE14.
- A detailed phylogenetic analysis on acyl-ACP TE family TE14 resulted in the classification of 10 subfamilies, in which the previously characterized FatA- and FatB-type TEs were included. Most of the sequences in this family, especially bacterial TEs, haven't been functionally characterized, providing us a good source for identifying and studying novel TEs.
- Based on phylogenetic analysis combined with the fatty acid profile information of bacterial species, a total of 24 DNA sequences spanning across the 10 subfamilies were synthesized.
- Using *E. coli* K27, a strain that secretes synthesized fatty acids into the media, an *in vivo* assay has been set up and was used to determine the bioactivity of all 31 TEs that we have identified. This *in vivo* assay has the potential to be used for high-throughput screening of thioesterase mutants of novel function.
- Several bacterial TEs that can produce significant amounts of 4- and 6-carbon fatty acids were identified, including fjTE20, which produced up to 20 mol% C6 fatty acid and 15 mol% C4 fatty acid (Figure 1). Another novel TE was found to produce the methyl ketone, 2-tridecanone.
- The fjTE20 and two other TEs (fjTE1 and fjTE6) that produce mainly C8 and C14 fatty acids, respectively were transferred to thrust II for production tests in *E. coli* and yeast.
- A directed evolution system has been designed for acyl-ACP TE. Random mutants were generated by error-prone PCR and screened on plates containing neutral red, a pH indicator that changes color due to fatty acid production. Fatty acid content of mutants was analyzed by GC-

MS (Figure 2). Using this approach, a mutant was identified (fjTE20-MT9) with a 2-fold increase in activity on 6-carbon fatty acid (~37 mol%), as compared with wild-type enzyme (fjTE20). fjTE20-MT9 and its progenitor, fjTE20 have been transferred to Thrust II researchers Ka-Yiu San and Nancy DaSilva for characterization and optimization in *E. coli* and *S. cerevisiae*, respectively.

- A Cys residue has previously been predicted as part of the catalytic triad of plant acyl-ACP TE (Yuan et al., J Biol Chem, 1996). Based on multiple sequence alignment and structure modeling, we proposed a different catalytic residue, Glu. Site-directed mutagenesis was performed on one coconut TE to verify this prediction. Mutating Glu to Asp or Ala dramatically decreased TE activity, whereas mutating the previously proposed active site Cys to Ser or Ala did not greatly impact catalytic activity, suggesting Glu is more likely to be the catalytic residue rather than the previously proposed Cys.

From 3/1/2011 to 2/29/2012:

- Previously, three TEs from coconut and three from *Cuphea viscosissima* were characterized. Another 25 TEs were selected for characterization from the public database based on phylogenetic analysis. The *in vivo* activities of these TEs were determined in *E. coli* K27. Novel TEs that can produce short chain FAs, unsaturated FAs, or methylketones were identified (Jing, Cantu et al, BMC Biochemistry, 2011).
- Acyl-ACP TEs fjTE10 (that expresses C8 specificity) and fjTE20 (that expresses C6/C4 specificity) were over-expressed in *E. coli* and purified in large quantities. High-throughput crystallization screening was performed with both proteins. As a result, initial crystallization conditions for fjTE10 and potentially favorable conditions for fjTE20 have been identified (Figure 3).
- To study the kinetic characteristics of acyl-ACP TEs, a new *in vitro* enzymatic assay has been developed. *O*-phthaldialdehyde (OPA) was used to derivatize the thiol on the prosthetic group of *holo*-ACP. After hydrolysis of acyl-ACP by a TE, the resulting *holo*-ACP can be measured with a fluorescence spectrophotometer. Due to the high sensitivity of this method, only small amounts of substrates are required to perform the assay (Figure 4). In addition, because this is a fluorescence-based assay it is amenable to high-throughput measurements.
- To identify residues that determine substrate specificity of acyl-ACP TEs, ~20 chimeric TEs have been generated and characterized from two plant TEs, CvFatB1 and CvFatB2, each of which show different substrate specificities. Specifically, these TEs share >70% amino acid identity, but CvFatB1 is C8/C10 specific, and CvFatB2 is C14/C16 specific. By comparing the sequences and substrate specificities of these chimeric TEs, the region that determines substrate specificity has been identified. In complementary work, phylogenetic inference and ancestral sequence analyses have been performed in collaboration with Dr. Xun Gu (Iowa State University) and have identified 9 key residues potentially related to substrate specificity. Interestingly, these 9 residues reside within the region identified by comparing substrate specificities conferred by our chimeric enzymes. Site-directed mutagenesis is being used to verify these predictions. Our current results show that at least some of these residues do affect the substrate specificity of acyl-ACP TE.

Other Relevant Work

Novel acyl-ACP thioesterases obtained in this research are currently being used to engineer FAS metabolic pathway in *E. coli* and yeast for producing short chain fatty acids in the Thrust 2 labs of

Ka-Yiu San and Nancy DaSilva, respectively.

In a complementary effort, the Reilly group has identified and classified 16 families of acyl carrier proteins (ACPs) from sequences currently available in public databases. Normal mode analysis was conducted to compare dynamic structures and computational methods have been used to predict the three-dimensional structures of ACPs for which structures are not available (Cantu et. al., pending final acceptance in Protein Sci). Continued work in this area will address how the acyl chain "fits" within the ACP protein structure and how TEs (and other enzymes) gain access to the acyl-chain within the acyl-ACP structure for catalysis.

Plans for the Next Year

- Using the new *in vitro* enzymatic assay, determine the kinetic characteristics of acyl-ACP TEs, including the TEs purified for crystallization and the mutants generated to verify both the catalytic residues and residues that affect substrate specificity.
- Perform directed evolution on acyl-ACP TEs that produce short chain fatty acids to increase the activity and specificity on short chain substrates. Use GC-MS to screen for other novel TEs.
- Optimize crystallization conditions for fjTE10 and fjTE20; purify other acyl-ACP TE proteins (e.g. CvFatB1 and CvFatB2) for crystallization screening.
- Set-up for solving the crystal structures of several acyl-ACP TEs (e.g., fjTE10 , fjTE20, CvFatB1, and CvFatB2), which have diverse substrate specificities. Understand the catalytic mechanism of TE and the structural basis that confers TE substrate specificity.
- Rationally design acyl-ACP TEs with desired activities, such as TEs specific for short chain fatty acids and TEs that produce substituted fatty acids.

Expected Milestones and Deliverables

- Identification of residues that determine the substrate specificity of acyl-ACP TE by year 2012
- Crystal structures of several acyl-ACP TEs with different substrate specificities by year 2013
- Understanding the structure-function relationship of acyl-ACP TE by year 2013
- Acyl-ACP TEs with rationally designed activities by year 2013

Member Company Benefits

This project has identified novel biocatalysts that can hydrolyze the fatty acid from acyl-ACP, terminating the fatty acid elongation process at considerably shorter chain lengths than normal, specifically at 4-, 6-, 8-, 10 and 12- carbon chain lengths. In addition, novel TEs that can produce unsaturated FAs or methylketones have also been identified. These novel enzymes have benefits for companies that have products in the detergent and surfactant markets, and with bioenergy companies. We have established collaborations with two of our partner companies. We have transferred seven of these novel TE sequences to our partner companies. A member company is evaluating one TE's utility relative to their host biocatalyst to produce 8- and 10-carbon fatty acids. We are exploring the possibilities of establishing a start-up company around this TE technology, and this is being conducted in collaboration with our company members.

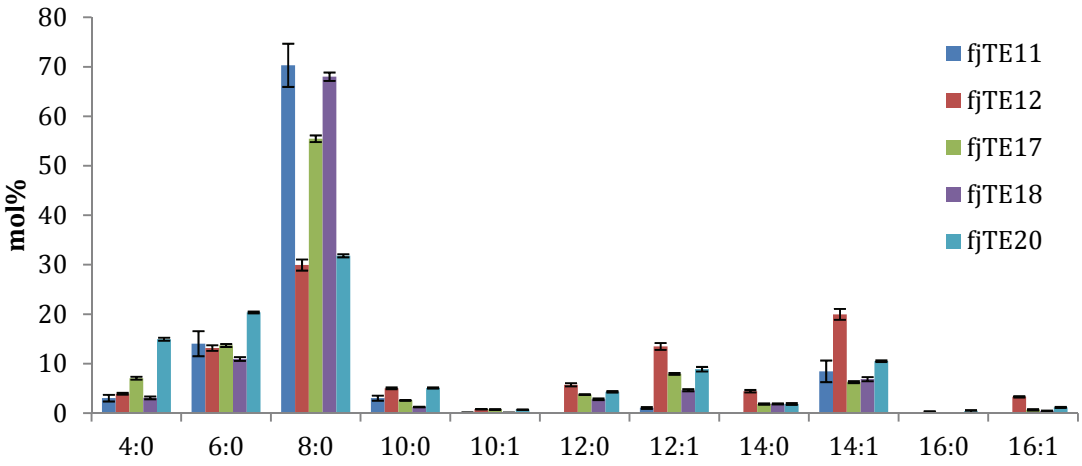

Figure 1. Fatty acid composition of *E. coli* K27 cultures over-expressing TEs. Each TE was expressed in *E. coli* K27, which contains a mutation in the fadD gene and secrets free fatty acids. The free fatty acids that accumulated in growth culture were analyzed with GC-MS and the mol percentage of each fatty acid was calculated.

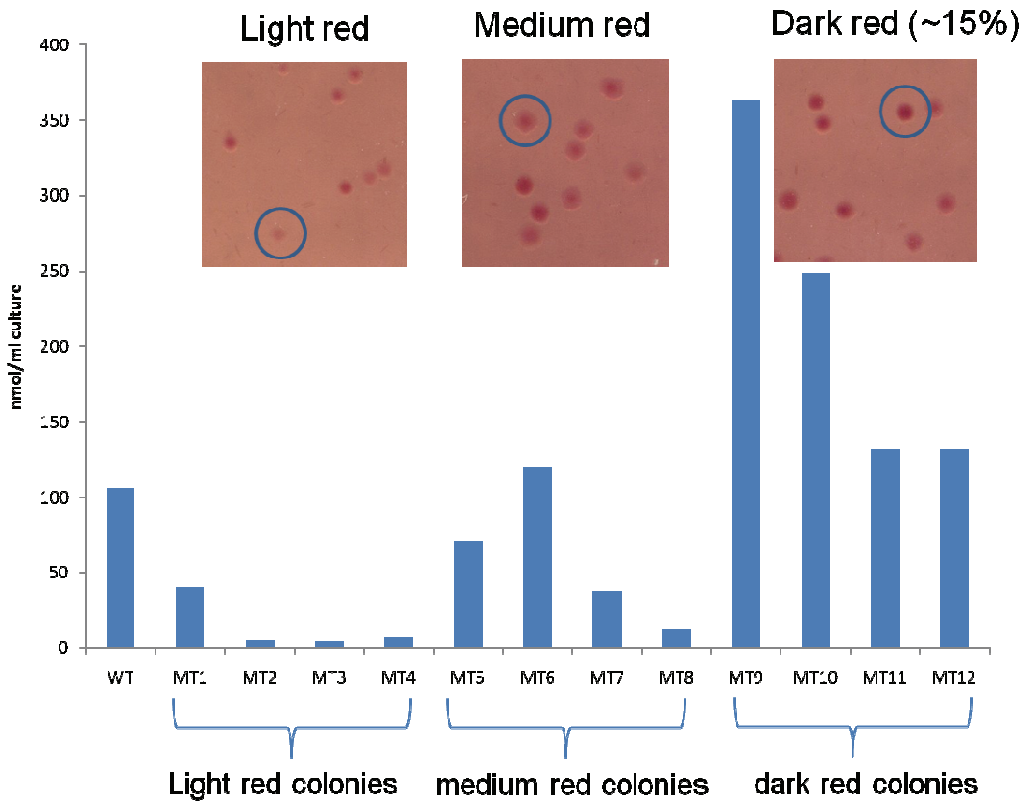

Figure 2. Identification of directed evolution mutants that produce different amounts of fatty acid. Mutants producing high concentrations of fatty acid were identified by staining with Neutral red, a pH indicator that changes to red color at pH < 6.8. Colonies of different colors were analyzed by GC-MS. Results demonstrated that there is a correlation between fatty acid production and color intensity of colonies. A mutant (MT9) with much higher activity than wild type enzyme was identified.

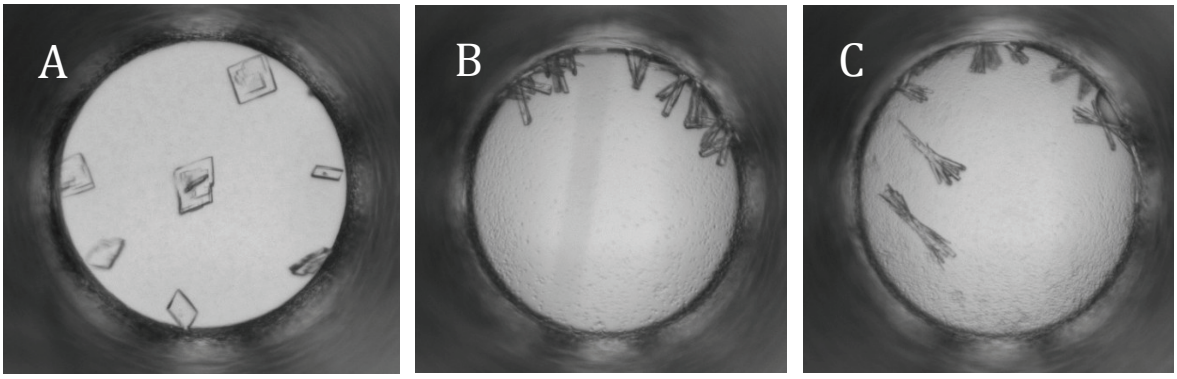

Figure 3. Initial crystallization hits of fjTE10 identified from high-throughput crystallization screening (a total of 1536 conditions). A, B, C, microscope images of crystals in 3 different buffers.

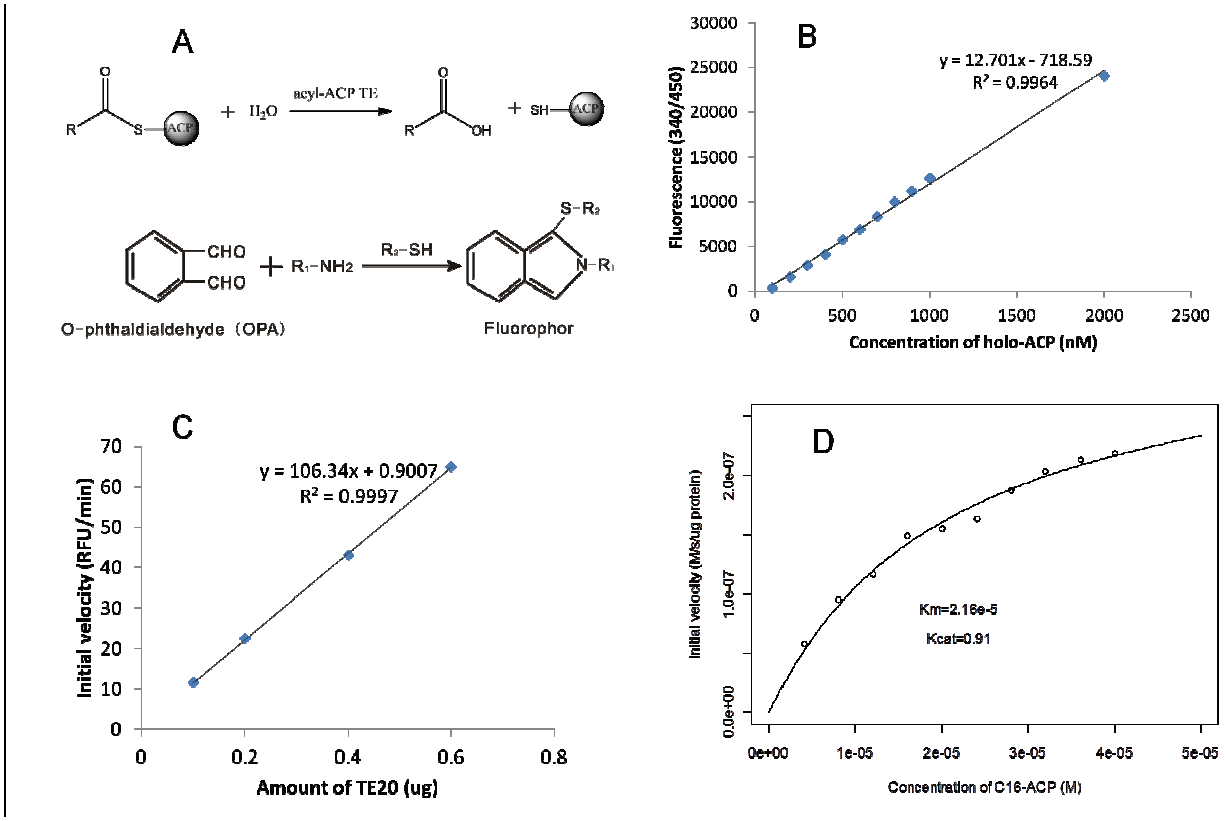

Figure 4. Development of a new *in vitro* enzymatic assay for acyl-ACP TEs. A, The reaction of acyl-ACP TE and the derivatization of thiol with OPA; B, Standard curve for quantification of holo-ACP after OPA derivatization; C, Initial velocity of different amounts of fjTE20 catalyzing the hydrolysis of 10 μM C16-ACP; D, Determination of the kinetic parameters of fjTE20 on the substrate C16-ACP.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems – Database Construction
[*This is an activity that supports all projects in Thrust 1, but it is being included here as an addendum to project T1.6.*]

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Peter Reilly	**02/17/2012**	**03/01/2011 to 02/29/2012**

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Project Leader: Peter J. Reilly
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Yingfei Chen (February 2009–present)
Undergraduate Students:
Michael Forrester (January 2011–present)
Jessica Foss (January 2011–August 2011)
Yu Gao (August 2011–December 2011)
Erin Kelly (June 2010–August 2011)
Hai Tran (January 2012–present)

Statement of Project Goals

We are constructing a database/webpage (ThYme, Thioester-active enzYmes, http://www.enzyme.cbirc.iastate.edu) that will contain all the amino acid sequences (primary structures) and three-dimensional structures (tertiary structures) of the enzymes in the eight enzyme groups involved in the fatty acid/polyketide synthesis cycle. Substrates of all of these enzymes contain thioester groups. Also included in ThYme will be the primary and tertiary structures of the acyl carrier proteins, which activate many of these substrates, and those of the thiolases and desaturases. We will use this information to write papers on the phylogeny and properties of these proteins.

Project's Role in Center's Strategic Plan

All center members will be able to access the primary and tertiary structures in ThYme. Since these data will be linked to many other databases, this should greatly simplify center members' tasks in gathering information on the enzymes on which they are working.

Fundamental Barriers and Methodologies

The largest barrier here is the sheer number of primary (270,000 at present) and tertiary structures (1,200 at present) that need to be gathered and ordered. In addition, new primary and tertiary structures are being discovered at an exponentially increasing rate (20% to 50% increase in numbers annually), and they must be gathered by us as they are discovered and placed in other

databases by other researchers. We have engaged the webpage development team in the ISU Information Technology Services to help in this effort. This has required extensive software coding that is essentially finished. This allows us to gather and to order data automatically, since there is far too much data to manage manually.

After gathering all primary and teriary structures within an enzyme group with the Basic Local Alignment Search Tool (BLAST) , we separate the sequences into families by their sequence differences using multiple sequence alignments with either Clustal or MUSCLE. With a few exceptions, members of different families are not related to each other, but instead are descended from different protein ancestors.

Achievements

We have completed classifying all eight enzyme groups [acyl-CoA synthases (ACSs), acyl-CoA carboxylases (ACCs), acyltransferases (ATs), ketoacyl synthases (KSs), ketoacyl reductases (KRs), hydroxyacyl dehydratases (HDs), enoyl reductases (ERs), and thioesterases (TEs)] whose sequences and structures we have gathered. The ThYme database is fully operational and is accessible to center members and to the general scientific public. A publication describing ThYme has appeared (Cantu, D. C., Y. Chen, M. L. Lemons, and P. J. Reilly. ThYme: A Database for Thioester-Active Enzymes. *Nucleic Acids Res.*, **39**, D342–D346 (2011)). In addition we have added acyl carrier proteins (ACPs) to ThYme, and we are about to add thiolases and desaturases to it also.

Acyl-CoA synthases: There are five ACS families, with the first (ACS1) having approximately 32,000 sequences and being composed of enzymes with many different names and with Enzyme Commission (EC) designations widely spread around the EC 6.2.1 group, which encompasses acid-thiol ligases. They are produced by bacteria, eukaryotes, and archaea (ordered in decreasing numbers produced). ACS2 has a few hundred sequences, mainly 6-carboxyhexanoate-CoA ligases and pimeloyl synthases (EC 6.2.1.14), produced by bacteria and archaea. ACS3 has a couple of hundred bacterial citrate (pro-3S)-lyase ligases (EC 6.1.2.22). ACS4 has about 1,500 bacterial, archaeal, and eukaryotal phenylacetate-CoA ligases (EC 6.2.1.30). ACS5 has about 9,500 bacterial, eukaryotal, and archaeal sequences, mainly succinate-CoA ligases (ADP-forming) (EC 6.2.1.5). Only ACS1, ACS4, and ACS5 have members with known tertiary structures. The work on this enzyme group is done. There may not be enough novel information here to justify a journal publication.

Acyl-CoA carboxylases: The ACCs are notable for having multi-domain structures, with the three main domains named for their roles [biotin carboxylases (BCs), biotin-carboxyl carrier proteins (BCCPs), and carboxyl transferases (CTs)]. BCs consist of one family of about 7,000 bacterial, eukaryotal, and archaeal sequences. BCCPs have about 4,500 bacterial, eukaryotal, and archaeal sequences, all in one family. There are two families of CTs. CT1 has approximately 9,000 bacterial, eukaryotal, and archaeal sequences, while CT2 has about 2,000 bacterial and eukaryotal sequences. All four families have known tertiary structures. Some ACCs have other domains. Furthermore, often other enzymes are found on the same protein chain, meaning that ACCs are not only multi-domain but are also part of multi-enzyme complexes. Although we have collected all primary and tertiary structures of enzymes in this group, we are still trying to sort out all the different ways that its members are put together. A quite complete review of the ACCs has recently appeared, probably limiting us to an article on BCCP classification.

Acyltransferases: ATs reversibly transfer CoA and acyl carrier protein (ACP) moieties linked by thioester bonds to acyl groups. There is only one AT family, consisting of approximately 9,000 primary structures, mainly in EC 2.3.1.39 (malonyl CoA-ACP transacylases). Nearly all ATs are produced by bacteria and eukaryota, with only a few archaeal producers listed. Many AT tertiary structures are known.

Ketoacyl synthases: There are five KS families, with KS1 through KS4 having some primary structures in common. KS1, KS2, and KS4 have very similar tertiary structures and therefore are in the same clan and appear to be descended from a common distant ancestor. KS1 has approximately 4,000 primary structures representing two enzymes, β-ketoacyl-[ACP] synthase I (EC 2.3.1.41) and β-ketoacyl-[ACP] synthase III (EC 2.3.1.180) produced by bacteria only. KS2 has about 500 sequences and no known tertiary structure, and to decide whether it is in the same clan as KS1, KS3, and KS4 will require its tertiary structure to be predicted by homology modeling. All KS2 members are produced by eukaryota; they have been given many names and several EC numbers. KS3 is a very large family, with about 17,000 sequences produced by bacteria, eukaryota, and a few archaea, and having many names and EC numbers. KS4 has somewhat over 2,500 eukaryotal and bacterial primary structures, many of them chalcone synthases or naringenin-chalcone synthases (EC 2.3.1.74). KS5 has almost 1,500 sequences, all produced by eukaryota and being fatty acid elongases. This family has no known tertiary structure and seems to be quite different than the other four KS families.

Because of the importance of the KSs, we have split the five families into subfamilies. As mentioned previously, all members of a family have related primary structures, but members of one subfamily have sequences that are statistically different from those of another. KS1, KS2, KS4, and KS5 have 12, 10, 10, and 11 subfamilies, respectively. KS3 has 14 manually separated groups, the different treatment warranted by its size.

This work was finished in spring 2011, and it led to a journal article: Chen, Y., E. E. Kelly, R. P. Masluk, C. L. Nelson, D. C. Cantu, and P. J. Reilly. Structural Classification and Properties of Ketoacyl Synthases. *Protein Sci.*, **20**, 1659–1667 (2011).

Ketoacyl reductases: There are four KR families. KR1 is the largest family among the eight enzyme groups in the fatty acid/polyketide synthesis cycle, having approximately 51,000 sequences produced by bacteria, eukaryota, and archaea. Given the size of this family, its members have many names and EC numbers, but many are 3-ketoacyl-[ACP] reductases (EC 1.1.1.100). KR2 has over 2,500 bacterial, eukaryotal, and archaeal primary structures, many of them 3-hydroxyacyl-CoA dehydrogenases (EC 1.1.1.35), named after the reverse reaction. KR3 has a couple of hundred eukaryotal and bacterial 3-ketoacyl-[ACP] reductases that are part of fatty acid synthases. KS4 has about 3,500 bacterial and eukaryotal enzymes that are found with other enzymes in polyketide synthases. All four KR families have members with known tertiary structures.

Hydroxyacyl dehydratases: There are a great number of different dehydratases/hydratases, but only four of them act on substrates with thioester groups. Enoyl-CoA hydratases (EC 4.2.1.17) are found in HD1 and HD2, unrelated by sequence similarity. HD1 has fewer than 1,000 members, nearly all produced by bacteria, with only a few produced by archaea and eukaryota. HD2 has over 7,000 primary structures, found in bacteria, eukaryota, and a few archaea. The near 1,000 HD3 sequences, produced by eukaryota and bacteria, are mainly 3-hydroxypalmitoyl-[ACP] dehydratases (EC 4.2.1.61), found in multienzyme fatty acid synthases, plus peroxisomal multi-

functional enzymes (EC 4.2.1.107). HD4 is comprised of a few hundred eukaryotal and bacterial 3-hydroxypalmitoyl-[ACP] dehydratases, also found in fatty acid synthases. HD5 consists of about 700 3-hydroxydecanoyl-[ACP] dehydratases (EC 4.2.1.60) produced almost solely by bacteria, while HD6 has over 2,500 mainly bacterial undesignated hydroxyacyl-[ACP] dehydratases (EC 4.2.1.–]. All six families have at least one member with a known tertiary structure.

Enoyl reductases: There are five ER families. ER1 has been absorbed into KR1, as the BLAST query sequence that produced this family appears to have actually been a KR. ER2 members are enoyl-[ACP] reductases (EC 1.3.1.9) produced by bacteria and some eukaryota. There are over 1,500 of them. The couple of hundred enoyl-[ACP] reductase members of ER3 are produced by bacteria and eukaryota and are nearly all found in fatty acid synthases. The over 2,000 ER4 members are produced by bacteria and eukaryota and a few archaea and appear to be mainly parts of polyketide synthases. The few hundred eukaryotal and bacterial ER5 members are mainly *trans*-2-enoyl-CoA reductases (EC 1.3.1.38). Finally, ER6 is composed of almost 7,000 bacterial, eukaryotal, and archaeal 2,4-dienoyl-CoA reductases (EC 1.3.1.34). All families have members with known tertiary structures.

We are within a month or two of submitting a manuscript to *Protein Engineering, Design, and Selection* describing the KR, HD, and ER families. Its authors will be David C. Cantu, Tingsong Dai, Zachary S. Beversdorf, Jessica L. Foss, and Peter J. Reilly, and its title will be Structural Classification and Properties of Ketoacyl Reductases, Hydroxyacyl Dehydratases, and Enoyl Reductases.

Thioesterases: There are 25 thioesterase families, in general not related by sequence similarity. Thirteen families are composed of acyl-CoA hydrolases, along with six families of acyl-ACP hydrolases, one family of protein-palmitoyl hydrolases, one family of protein-acyl hydrolases, two families of glutathione hydrolases, and two families that are yet unclassified. Only 12 of the 27 enzymes classified by function into EC 3.1.2.– are represented. Twelve of these families can be grouped into four clans by their slight similarity in primary structure but strong similarity in tertiary structure, with two clans having members with HotDog crystal structures, and with the other two clans whose members have α,β-hydrolase structures. A manuscript on this work has appeared (Cantu, D. C., Y. Chen, and P. J. Reilly. Thioesterases: A New Perspective Based on Their Primary and Tertiary Structures. *Protein Sci.*, **19**, 1281–1295 (2010)).

We have used multiple sequence alignments made with ClustalX or MUSCLE to further study TE14, because it contains enzymes that act on substrates containing short fatty acid chains. TE14 has ten subfamilies, four whose members are produced by plants and six whose members are produced by bacteria, with the members of each subfamily related to each other by their very similar sequences, but yet showing statistically significant differences from one subfamily to the next. We had hoped that thioesterase-catalyzed hydrolysis would liberate fatty acids and other molecular building blocks of the number of carbon atoms (4–8) desired by CBiRC, and that has occurred. We have published a manuscript based on our subfamily work as well as on data from experiments designed from our subfamily data and gathered by the Nikolau group (Jing, F., D. C. Cantu, J. Tvaruzkova, J. P. Chipman, B. J. Nikolau, M. D. Yandeau-Nelson, and P. J. Reilly. Phylogenetic and Experimental Characterization of an Acyl-ACP Thioesterase Family Reveals Significant Diversity in Enzymatic Specificity and Activity. *BMC Biochem.*, **12**, 44 (2011).

Acyl carrier proteins: All ACP primary and tertiary structures were gathered into the ThYme database. They are classified into 16 families by amino acid sequence similarity, with members of the different families having sequences with statistically highly significant differences. These classifications are supported by tertiary structure superposition analysis. Tertiary structures from a number of families are very similar, suggesting that these families may come from a single distant ancestor. Normal vibrational mode analysis was conducted on experimentally determined freestanding structures, showing greater fluctuations at chain termini and loops than in most helices. Their modes overlap more so within families than between different families. The tertiary structure of an ACP family that lacked any known structures was predicted as well. We are about to submit a revised version of a manuscript to *Protein Science.* Its authors are David C. Cantu, Michael J. Forrester, Katherine Charov, and Peter J. Reilly and its title is Acyl Carrier Protein Structural Classification and Normal Mode Analysis.

Thiolases and desaturases: Although thiolases and desaturases are not part of the fatty acid and polyketide synthesis cycles, they act on these compounds when they are activated through thioester bonds with ACP or CoA, and therefore they are candidates for inclusion in the ThYme database. We have gathered all their primary and tertiary structures, finding two families of thiolases and one family of desaturases by BLAST, multiple sequence alignment, and tertiary structure superposition, as with the other proteins that we have added to ThYme. We probably will not be able to write an article on the thiolases, as a very complete review on their phylogeny appeared in 2005. Whether we can publish our work on desaturases is not yet clear.

Other Relevant Work

There are other databases similar to ThYme, specifically ones for enzymes that are active on carbohydrates (CAZy) and peptidases (MEROPS). These gather thousands of hits from researchers around the world, and we expect that ThYme will also.

Plans for the Next Year

March 2012–February 2013:

1) Add thiolase and desaturase primary and tertiary structures to ThYme.

2) Submit manuscripts on KR/HD/ER phylogeny (if not already done), BCCP phylogeny, and perhaps ACC and desaturase phylogenies to peer-reviewed journals.

3) Determine by homology modeling the tertiary structure of KS2 members, and through that whether KS2 members are part of the same clan as KS1, KS3, and KS4 members.

4) Determine by homology modeling the tertiary structure of KS5 members (a much harder project than the previous one).

5) Submit a manuscript on quantum mechanics/molecular mechanics simulation of TE8-catalyzed hydrolytic reaction, and perhaps that of another fatty acid/polyketide synthesis enzyme.

6) Construct a webpage that allows BLAST data to be converted into a more usable format identical or similar to that of ThYme, based on the scripts that we have written to construct ThYme, and submit a small manuscript describing the webpage.

7) Continue working on the simulation of ACP-assisted delivery of growing fatty acid chains to the

active sites of fatty acid cycle synthesis enzymes.

8) Assist other CBiRC researchers in deciding which members of different enzyme groups to experimentally investigate.

Expected Milestones and Deliverables

Our expected milestones and deliverables are mainly listed above. The ThYme database will continue to grow indefinitely as new sequences and structures are automatically added to it. Its continued maintenance is a long-term commitment, as such resources cannot easily be abandoned without damage to the university's reputation and to researchers, here and elsewhere, who depend on it.

Member Company Benefits

CBiRC's industrial members will have full access to ThYme and will be able to use it to gain information on the various enzymes and proteins tabulated in it. It is impossible to estimate the number of person-hours and the production costs saved, or the market impact, but databases such as these save very large percentages, much greater than 50%, of the time needed for gathering information.

Commercialization / Technology Transfer

We have already worked out a protocol so that a CBiRC-affiliated company has downloaded all of ThYme. We are prepared to assist other companies, within and outside of CBiRC, to download all or parts of ThYme.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.1A -** Strain Construction and Optimization in *E. coli*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ka-Yiu San	**02/17/2011**	**03/01/2010 to 02/29/2011**

ERC Team Members

Project Leader: Ka-Yiu San, Rice University
Other Faculty: Ramon Gonzalez, Rice University; Laura R Jarboe, ISU
Research Scientists: Mai Li and Xiujun Zhang, Rice University
Graduate Student: John Park, Rice University; Liam Royce, ISU

Statement of Project Goals

The goal of the project is to develop metabolic engineering tools to design and construct efficient *Escherichia coli* strains for high level production of biochemical intermediates from glucose.

Project's Role in Center's Strategic Plan

The project plays a central role in bridging the other two research thrusts. Specifically, the project focuses on constructing efficient microbial systems to produce biochemical intermediates, which will be used in Thrust 3, using knowledge and materials from Thrust 1. Specifically, genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the product fatty acids from Thrust 2 will serve as precursors for the synthesis of α-olefins by Thrust 3.

Fundamental Barriers and Methodologies

Successful development of efficient strains for high level production of biochemical intermediates from glucose requires several issues to be addressed. The first challenge is to introduce new functional pathways into *E. coli* to produce the targeted product. Since most of the genes involved in these pathways are from plants, the expression of biologically active enzymes in *E. coli* may require additional effort. The enzymes might have to be modified in order to function efficiently in *E. coli*. Furthermore, the production strain must be designed to be able to channel cellular resources, such as carbon precursors, cofactors and energy, for the synthesis of the desired product. In this project, molecular biology and metabolic engineering techniques (including co-factor engineering) will be developed and used to overcome these challenges. More importantly, strain development is an iterative process, knowledge learned from other projects, such strain characterization and omics studies, will be used to provide insight in designing additional strains with improved performance.

Achievements

1) Carboxylic acid

1A) Construction of plasmids carrying modified acyl-ACP thioesterases (TE): The enzyme acyl-ACP thioesterase (TE) plays an important role in the biosynthesis of free fatty acid as it facilitates the release of free fatty acid from the carrier protein. Most current and past studies have been focused on the substrate specificity of the acyl-ACP thioesterase; few studies, however, have been reported concerning the improvement of the thioesterase activity, especially in *E. coli*. We aimed to increase the accumulation of free fatty by designing and constructing modified acyl-ACP thioesterases that exhibit improved enzyme properties. The positive results in fatty acid accumulation from the overexpression of a castor bean thioesterase (including its leading sequence (LS)) suggest that the leading sequence might have a stabilization effect of the LS-TE fusion protein in *E. coli*. A similar construct, pXZR16, was constructed with an acyl-ACP thioesterase from *D. butyracea*. For comparison purpose, another plasmid, pXZT16, was constructed using a commonly used thioredoxin sequence. Simulation results from David Cantu of Peter Reilly's group (Thrust 1) suggest M16 has a helix (colored green) that is not present in R16 and T16 (Fig. 1A-1). Further analysis indicates the helix in the N-terminal region of M16 might be more hydrophobic and removing that helix might make M16 more soluble. Based on this hypothesis, two more constructs, pXZM16D37 and pXZR16D37, were created. In summary, two approaches were examined to improve the expression of functional acyl-ACP thioesterase: 1) through the construction of a fusion protein to increase protein solubility; and 2) to delete the hydrophobic segment in the N-terminal region of the acyl-ACP thioesterase (based on the results from simulation study by David Cantu of Thrust 1). A list of constructs is shown below.

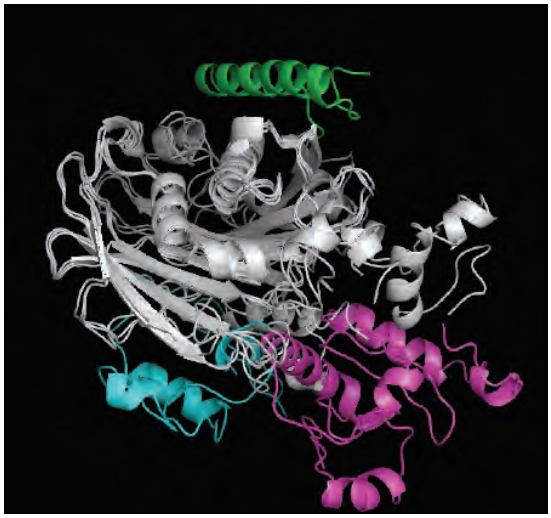

Fig 1A-1 Image showing three structures superimposed. They all have the same core area (colored in grey for the three), but different N-terminal domains: Green -M16; blue - R16 and purple - T16 (Image created by David Cantu (Thrust 1).

pTrc99A	Cloning vector
pXZM16	pTrc99A carries a mature acyl-ACP thioesterase from *D. butyracea*
pXZR16	pTrc99A carries a modified acyl-ACP thioesterase from *D. butyracea*
pXZT16	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris* (with a commercial Thioredoxin sequence (pThioHisB, Invitrogen)
pXZM16D37	Based on pXZM16 but with the deletion of the hydrophobic region
pXZR16D37	Based on pXZR16 but with the deletion of the hydrophobic region

1B) **Overexpression of acyl-ACP thioesterase together with gene(s) involved the fatty acid synthesis cycle.** Several plasmids were constructed to test the hypothesis that selectively over-expresses gene(s) involved in the fatty acid synthesis cycle will increase fatty acid production. A transcription activator, *fadR*, which activates the expression of *fabA* and *fabB*, was cloned and placed after the TE gene from castor bean. In addition, in order to further examine the effect of individual fatty acid gene, two more constructs, pXZ18A and pXZ18Z, where the fabZ or fabA gene was cloned and placed after the TE gene from castor bean (see list below).

pXZ18Z	pTrc99A carries an acyl-ACP thioesterase from castor bean and *fabZ* from *E. coli*
pXZ18A	pTrc99A carries an acyl-ACP thioesterase from castor bean and *fabA* from *E. coli*
pXZ18fadR	pTrc99A carries an acyl-ACP thioesterase from castor bean and *fadR* from *E. coli*

1C) Construction of plasmids carrying thioesterases for shorter chain length carboxylic acid production. Various plasmid constructs tragetting shoter chain length fatty acids (C-8, octanoic acid) were made. The acyl-ACP thioesterase from two plants (*Cuphea palustris and C. hookeriana*) was chosen for this study. Various variants of the mature acyl-ACP thioesterases were consturcted based on a pantent pending technology. A partial list of the plasmid constructs is shown below.

Name	Description
pXZCP	pTrc99A carries an acyl-ACP thioesterase from *Cuphea palustris*
pXZmCP	pTrc99A carries a mature acyl-ACP thioesterase from *C. palustris*
pXZmCPI	pTrc99A carries a modified mature acyl-ACP thioesterase from *C. palustris*
pXZCH	pTrc99A carries an acyl-ACP thioesterase from *C. hookeriana*
pXZmCH	pTrc99A carries a mature acyl-ACP thioesterase from *C. hookeriana*
pXZCP80	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pXZCP88	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pXZCH80	pTrc99A carries a modified acyl-ACP thioesterase from *C. hookeriana*
pXZCP	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pXZCH88	pTrc99A carries a modified acyl-ACP thioesterase from *C. hookeriana*
pXZCP8029	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pXZCP8829	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pXZCH8029	pTrc99A carries a modified acyl-ACP thioesterase from *C. hookeriana*
pXZCH8829	pTrc99A carries a modified acyl-ACP thioesterase from *C. hookeriana*
pXZCP8060	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pXZCP8860	pTrc99A carries a modified acyl-ACP thioesterase from *C. palustris*
pCBiRC0	pTrc99A carries an acyl-ACP thioesterase TE0 *(C. palustris*) from Thrust 1
pCBiRC088	pTrc99A carries a modified acyl-ACP thioesterase TE0 *(C. palustris*) from Thrust 1

1D) Host strain construction – Central pathway manipulations: Several mutant strains were created based on hypothesis that a TCA cycle mutant strain might reduce the carbon flow through the cycle and hence might increase the acetyl-CoA availability and divert higher carbon fluxes to the fatty acid synthesis cycle. The positive results from the *sucC* mutant strain (MLK163) were very encouraging. We have constructed two more mutant strains with mutations (gene knock out) in the TCA cycles (*fumAC*$^-$, *gltA*$^-$) for comparison purposes. We further constructed several mutant strains (*ptsG*$^-$, *glk*$^-$, *pykF*$^-$, *pfkA*$^-$) to test the hypothesis that a mutant strain with reduced glucose uptake rate or reduced rate in the glycolysis pathway might allow a higher flow of carbon flux to the fatty acid synthesis and hence increase the fatty acids/glucose yield. Finally, three more triple mutant strains were constructed to test the combination effect. These MG1655 based mutant strains are listed below.

Name	Relevant Genotype
MLK163	MG1655(Δ*fadD*Δ*sucC*)
MLK193	MG1655(Δ*fadD*Δ*fumAC*)
MLK195	MG1655(Δ*fadD*Δ*gltA*)
MLK189	MG1655(Δ*fadD*Δ*glk*)
MLK190	MG1655(Δ*fadD*Δ*ptsG*)

MLK191	MG1655(Δ*fadD*Δ*pfkA*)
MLK192	MG1655(Δ*fadD*Δ*pykF*)
MLK194	MG1655(Δ*fadD*Δ*sucC* Δ*ptsG*)
MLK181	MG1655(Δ*fadD*Δ*sucC* Δ*glk*)
MLK182	MG1655(Δ*fadD*Δ*sucC* Δ*pfkA*)

1E) Host strain construction – Fatty acid synthesis pathway manipulations: Several mutant strains were constructed to study the effect of inactivation of two transcription factors, FadR and FabR, involved in the regulation of the fatty acid biosynthesis/degradation pathways. It is hypothesized that inactivation of the transcription factor *fabR*, which represses the expression of *fabA* and *fabB*, will lead to increased expressions of *fabA* and *fabB*. It is also speculated that the inactivation of the transcription factor *fadR*, which represses the expression of *fadA* and *fadB* but activates the expression of *fabA* and *fabB*, will have the opposite effect.

Name	Relevant Genotype
MLK211	MG1655(Δ*fadD* Δ*fabR*)
MLK212	MG1655(Δ*fadD*Δ*sucC*Δ*fabR*)
MLK225	MG1655(Δ*fadD* Δ*fadR*)
MLK213	MG1655(Δ*fadD*Δ*sucC*Δ*fadR*)
MLK227	MG1655(Δ*fadD* Δ*fadR* Δ*fabR*)

Other Relevant Work

Since the biosynthesis of fatty acid requires significant quantity of the cofactor NADPH and acetyl-coA (for ex., each fatty acid elongation cycle requires two molecules of NADPH), results and knowledge from another project aiming to design and construct efficient strains with increased NADPH availability for chiral compound production may be useful in the current project to increase fatty acid levels.

Plans for the Next Year

Further fine-tuning of the host strains and expression vectors will be carried out in the coming years. Furthermore, the design, construction and characterization of more advanced modified acyl-ACP thioesterases leading to higher specificity and productivity will be pursued. The design of the second generation production strains will be based on the characterization and omics studies. In addition, single plasmid carrying multiple genes will also be constructed to study the effect of introducing multiple genes into the system (integration into the chromosome if necessary). These multiple genes constructs will be performed after initial proof-of-concept experiments with multiple compatible plasmids each carrying a single gene. Finally, efforts will also be put on targeting the production of shorter chain length carboxylic acids and improve the purity of the carboxylic acids (i.e., with one predominant chain length rather than a mixture). As such further development of new and more efficient acyl-ACP thioesterases (in terms of activity and specificity) as well as strains is necessary.

Specifically, we plan to: 1) built on current framework to integrate various genetic and metabolic manipulations to further increase the product titer; 2) perform further metabolic engineering studies to improve current strain to increase product/glucose yield; 3) develop new strains based current framework to produce shorter chain length carboxylic acids efficiently; 4) design, construct and characterize more efficient thioesterases for efficient production of shorter chain length carboxylic acids; 5) perform characterization studies to gain insight into the mechanisms leading to efficient strain and thioesterase constructs; 6) incorporate mutations that confer fatty acid tolerance into the fatty-acid producing strain (dependent on identification of those mutations, described in bioinformatics report; 7) address metabolic bottlenecks identified in the simultaneous flux/transcriptome/proteome study.

Expected Milestones and Deliverables

The deliverables will be strains and vectors for the expression of biologically active enzymes for short/medium chain fatty acid biosynthesis. We will improve *E. coli* fatty acid productivity through strain design and construction aiming to attain higher titer, yield and production rate. In addition, we will design, construct and characterize modified acyl-ACP thioesterases for the efficient production of purer free fatty acid at higher rates.

Member Company Benefits

The knowledge and constructs (plasmids, strains and acyl-ACP thioesterases) being developed in this project will be useful to member companies.

Commercialization / Technology Transfer

We are actively engaging with Center members and other companies, including start-ups, for potential licensing and commercialization of the carboxylic acid production technologies (five invention disclosures, 1 PCT application and 1 provisional application).

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.1B -** Strain Construction and Optimization in *S. cerevisiae*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Nancy A. Da Silva	**02/17/2012**	**03/01/2011 to 02/29/2012**

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Undergraduate Students: Christina Tran (UCI), Adam Dobson (REU student; University of Pittsburgh)

Statement of Project Goals

The goals of the work are to design and construct *Saccharomyces cerevisiae* strains for high-level production of carboxylic acids and pyrones from glucose, and to develop the necessary genetic tools to efficiently engineer the strains.

Project's Role in Center's Strategic Plan

The goal is to construct microbial strains to produce test bed chemicals, including carboxylic acids and pyrones. These test beds will provide opportunities to integrate all three research thrusts. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins, dienes, and other compounds by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

The synthesis of short chain fatty acids requires access of novel thioesterases (TEs) to the growing fatty acid chain. This is precluded in *S. cerevisiae* by the complex and closed structure of the native fatty acid synthase (FAS). To address this, we are introducing heterologous Type I and Type II FAS systems that allow enzyme access. The introduction of a second pathway also allows us to balance the synthesis of required fatty acids for cell viability and of the desired short-chain products. Synthesis of high levels of carboxylic acids and pyrones requires high-level production of active synthase enzymes, and sufficient precursor synthesis. Strains are being engineered and evolved for this purpose. In addition, the project requires the ability to efficiently construct and modify strains by introducing multiple genes. To address this, we have developed a robust set of expression vectors for metabolic pathway engineering in *S. cerevisiae*. Finally, a new project focuses on the development of strains and tools for the oleaginous yeast *Yarrowia lypolytica*, a promising yeast for

high-level polyketide synthesis.

Achievements

During the first three years of the Center, our efforts focused on the development of a *S. cerevisiae* toolkit for strain construction, and on the manipulation of fatty acid, methyl ketone, and pyrone synthesis in yeast. During the fourth year, we have (1) initiated work with the oleaginous yeast *Yarrowia lipolytica*, including developing the required tools for strain construction, (2) expanded the toolkit for *S. cerevisiae* metabolic engineering, (3) constructed strains for the expression of exogenous FAS systems in *S. cerevisiae* and introduced promising thioesterases for short chain fatty acid synthesis, (4) engineered strains for high level synthesis of pyrones, and (5) engineered *S. cerevisiae* for increased synthesis of required precursors.

(1) Development of strains and plasmid tools to address the basis of the reported higher levels of fatty acid production in *Yarrowia lipolytica*.

Based on the encouragement of the leadership, NSF site visit team and industrial members, we have undertaken work on one of the more completely described, but nonetheless understudied, oleaginous yeasts, *Y. lipolytica (Yl)*. The goal of this work is to develop a yeast that naturally stores lipids rather than polysaccharides as a metabolic model and to test whether it is more amenable to manipulation of acetate based precursors for production of hydrocarbon compounds of interest.

Y. lipolytica is not as amenable to molecular manipulation as *S. cerevisiae*. It has a lower frequency of recombination and requires longer regions of homology making gene disruptions or recombineering more challenging. Promoters, ORFs, and ARS/CEN sequences are not universally interchangeable. One of our goals was to develop a shuttle vector for expression of genes of interest in *Y. lipolytica*. This has now been accomplished. The vector is based on the pUC18 backbone and bacterial beta lactamase gene for ampicillin resistance. In addition, it contains the *Yl URA3* selectable marker and the ars18 *Yl* replicative sequence. The expression cassette into which various promoters, ORFs and terminators can be cloned, currently contains the *Yl TEF* promoter and *CYC1* terminator. The *TEF* promoter is flanked by restriction sites that allow replacement of the *TEF* promoter with other promoters of interest identified by RNA-seq. A GFP reporter is currently being introduced into the plasmid to facilitate testing of different promoters. We knocked out the *Yl URA3* gene creating a strain in which uracil-minus selection can be used to maintain the *URA3*-marked expression plasmid. Other modifications of the background are underway in order to enhance FA production.

(2) Expansion of toolkit for metabolic engineering in *Saccharomyces cerevisiae*

We previously completed construction of a toolkit of yeast pXP vectors to allow the combinatorial expression of metabolic genes in *S. cerevisiae*. A paper describing the initial vectors was published in *Yeast* in October 2011. We have expanded the vector set to include three inducible/repressible promoters: P_{GAL1}, P_{ADH2}, and P_{CUP1}. They are available as high- and low-copy plasmids, and are designed with reusable selectable markers that enable PCR amplification of cassettes, sequential chromosomal integration, and subsequent simultaneous excision of the markers. A new PCR-based integration strategy has also been designed to integrate tandem genes (with different promoters and terminators) into a single locus in order to optimize time and resources. The full set of vectors and expression loci characterized

facilitate rapid and systematic combinatorial expression of pathway genes for metabolic engineering, and have been used extensively in CBiRC projects 1B and 2B.

(3) Development of *S. cerevisiae* strains for synthesis of short chain fatty acids

To avoid the inherent limitations of the yeast FAS and to optimize short chain fatty acid production, we have investigated the expression of heterologous FAS systems (mammalian and *E. coli*) in *S. cerevisiae*. The non-native FAS systems allow access by the thioesterases required for short chain synthesis, enable easier optimization of the host by avoiding native regulatory control, and allow utilization of separate FAS systems for host cell requirements and product synthesis. Previously we showed that expression of active holo-mFAS was sufficient to complement a yeast *FAS2* knockout, allowing growth in the absence of exogenous fatty acid supplements. We integrated the auxiliary genes and engineered the yeast expression system for enhanced stability and robustness. This functional replacement is very promising as the mFAS can be used with thioesterases that allow short chain synthesis. We are currently developing and characterizing a strain for down-regulation of the native yeast FAS when heterologous mFAS expression is desired (Project 2B).

For the synthesis of short chain fatty acids, we have combined novel thioesterases (TEs) from Thrust I and two TEs from the literature with our new heterologous yeast systems. To test these TEs for short chain fatty acid synthesis, the TE domain was removed from the mFAS, and the new TEs are carried on plasmid vectors for testing. We previously showed the *in vitro* activity of the thioesterases expressed in yeast, and *in vivo* characterization studies are underway (Project 2B).

In addition, we have created a mutant mFAS with a short chain thioesterase domain by fusing the TE directly to the mFAS via a linker. This short chain TE domain replaces the native TE domain and allows the direct shuttling of acyl-CoA substrates from the adjacent ACP domain. This should increase short chain fatty acid synthesis as experiments indicate that release by the TE is limiting. *In vitro* and *in vivo* short chain fatty acid production will be monitored with the ADIFAB assay and GC-MS.

In parallel, we have focused on introducing the *Escherichia coli* fatty acid pathway as the separate proteins allow the greatest flexibility for manipulation. We have expressed all nine required genes in active form in yeast and confirmed activity with *in vitro* experiments (Project 2B). Integration of the genes into a single strain is well underway.

(4) Engineering of *S. cerevisiae* strains for high-level synthesis of pyrones

The enzyme 2-pyrone synthase (2-PS) from *Gerbera hybrida* (Thrust 1) has been cloned into pXP vectors under the control of the strong promoters P_{PGK1}, $P_{GAL1,}$ and P_{ADH2}. These plasmids have been transformed into our yeast host strains for analysis of pyrone synthesis levels. Two mutated polyketide synthases (*Medicago sativa* chalcone synthase, *Penicilin patulum* 6-methylsalicylic acid synthase) were cloned in order to compare pyrone synthesizing potential for this group of enzymes. Similar expression systems were constructed using six 2-PS mutants enzymes developed in the Noel laboratory (Thrust 1). Additional mutant enzymes have recently been provided by the Noel lab, and these are currently being expressed and analyzed for pyrone production.

Protease deficient strains were constructed by knocking out two common proteases, *PEP4* and

PRB1. Following construction of *pep4Δ*, *prb1Δ*, and *pep4Δ prb1Δ* strains, pyrone synthesis was evaluated using the plasmid constructs described. By combining the protease knockouts, optimum promoter, optimum expression system, and best 2-PS mutant, we have increased our pyrone titer 15-fold (to 1.1 g/L) and our yield 30-fold. Current efforts focus on further increasing titer and yield, and on developing an optimum expression system for use in minimal culture medium.

A computational approach using the OptKnock procedure included in the COBRA Toolbox (http://opencobra.sourceforge.net) was used to suggest metabolic engineering strategies to improve the strain's ability to produce pyrones, specifically precursor availability. The use of this toolbox provided pathways to be manipulated, which included both pyruvate metabolism and fatty acid synthesis in aerobic, glucose-fed cells. The construction of *pyc1Δ* and *pyc2Δ* strains, and the inhibition of fatty acid synthesis through the use of cerulenin, increased pyrone productivity (mg/L/OD) by as much as ~103% (Project 2B).

(5) Strain engineering for increased precursor synthesis

To engineer strains for increased production of fatty acids and related compounds, we previously knocked out specific regulatory and pathway genes, upregulated genes for the synthesis of important precursors, and evolved strains for high-level fatty acid synthesis and for increased resistance to short chain fatty acids. The key pathways are shown in Figure 1. These strains were evaluated by GC-MS for increased fatty acid levels (Project 2B) and characterized using DNA microarrays (Project 3B).

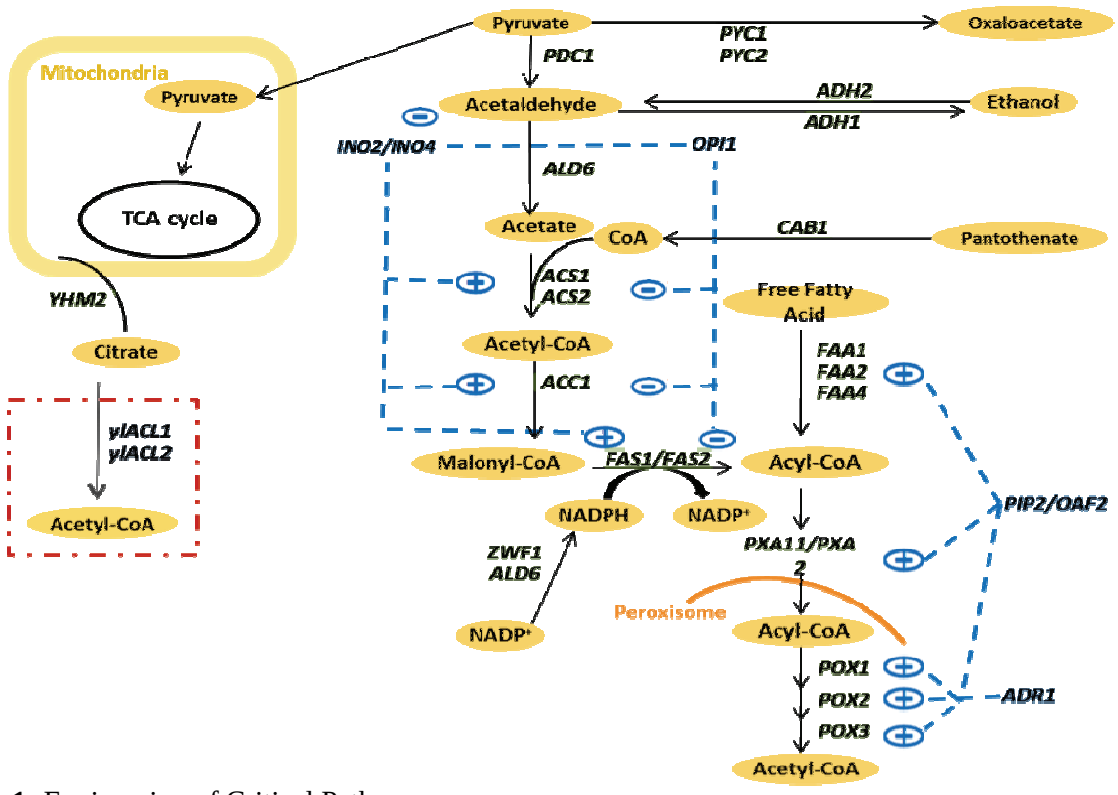

Figure 1: Engineering of Critical Pathways

The focus over the past year has been engineering strains carrying a combination of genes leading to increased production of precursors (e.g., CoA, acetyl-CoA, malonyl-CoA). Two

promoters have been incorporated and the strains are being evaluated for the increased synthesis of a model polyketide (6-MSA), fatty acids, and pyrones. Up to 4-fold increases in 6-MSA have been observed.

An alternative approach to increase the pool of acetyl-CoA utilizes a strategy from the oleaginous yeasts known to accumulate fatty acids. ATP Citrate Lyase (ACL) is an enzyme that cleaves cytosolic citrate into acetyl-CoA and oxaloacetate in oleaginous yeasts, e.g., *Y. lipolytica*. This reaction provides an influx of cytosolic acetyl-CoA, an important precursor for fatty acid biosynthesis. This enzyme is notably absent from non-oleaginous yeasts such as *S. cerevisiae*. It has been hypothesized that nitrogen limitation leads to an accumulation of citrate in the mitochondria, which passively diffuses into the cytosol. In oleaginous yeast, citrate is converted into cytosolic acetyl-CoA by ACL (comprised of two subunits encoded by ACL1 and ACL2), leading to an accumulation of fatty acids. If this is the case and ACL can be expressed in *S. cerevisiae*, it might be possible to elevate carboxylic acid synthesis by expressing ACL.

In order to test the dependence of fatty acid synthesis in the oleaginous yeast *Y. lypolytica* on ACL, the *ACL1* gene coding for the catalytic subunit of the ACL complex is being deleted. A knockout cassette has been constructed and will be transformed into *Y. lypolytica* and the mutant will be tested for the ability to accumulate lipid when grown on different test carbon sources. If ACL is responsible for the ability of oleaginous yeast to accumulate fatty acid, then it could be possible to confer this property on *S. cerevisiae* by introduction of *ACL1, 2* under control of the *Sc FAS2* promoter. As a first step in this test, we developed a strain in which the two genes required for production through pyruvate decarboxylation are knocked out (*PDC1* and *5*). These cells cannot survive on glucose as a carbon source because of inability to produce requisite levels of acetate. If *Yl* ACL is active, it should be possible to complement this deficiency by introduction of *Yl* ACL into the double deletion mutant. The epitope-tagged versions of *ACL1,2* are being introduced into *S. cerevisiae* in order to test expression and into the *pdc1,5* deletion strain in order to test for ability to complement that deficiency.

Other Relevant Work

Relevant similar work is also being conducted within CBiRC using *E. coli* as the model microbial system. In combination, the research will evaluate two promising microbial systems for the synthesis of the precursor compounds required for the Center's goals. To our knowledge, similar work on the use of heterologous FAS systems in yeast for the synthesis of short chain compounds is not taking place outside of this Center. Similarly, comparable work on pyrones is not underway.

Plans for the Next Year

Increase titer and yield of short chain fatty acids and pyrones:

Optimize yeast system for synthesis of short chain fatty acids. This work will involve selection of the optimum heterologous FAS system, removal of pathway bottlenecks, and modification of strains based on information from the Omics, Flux, and Bioinformatics groups.

Optimize yeast system for synthesis of pyrones. This work will involve selection of the optimum enzyme for use in the yeast host, removal of pathway bottlenecks, and modification of strains based on information from the Omics, Flux, and Bioinformatics groups.

Develop Y. lypolytica expression strains. The work will include the development of tools and strains for both short chain fatty acid and pyrone production. Transfer of pathways to *S. cerevisiae* may prove advantageous in this non-oleaginous yeast species.

Expected Milestones and Deliverables

Effective tools for metabolic engineering in yeast

Strains engineered with heterologous fatty acid synthase systems that allow manipulation of the synthesis pathway

Strains engineered for the high-level synthesis of limiting precursors

Strains engineered for high-level pyrone synthesis

Member Company Benefits

The benefits for the Center's industry members are the development of vectors and strains for the high-level synthesis of carboxylic acids and pyrones. In addition, strains with increased levels of the CoA precursors will be useful for a variety of products. Efficient metabolic engineering tools and methods developed will also be beneficial.

Commercialization / Technology Transfer

We have discussed collaborations with both Lesaffre and NCAUR for the pyrone research. Genes and vectors are being transferred to NCAUR (under a Materials Transfer Agreement) for studies in industrial yeasts. Further opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.2A -** Strain Characterization and Optimization in *E. coli*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ka-Yiu San	**02/17/2012**	**03/01/20011 to 02/29/2012**

ERC Team Members

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Research Scientists: Mai Li and Xiujun Zhang, Rice University
Graduate Students: John Park, Rice University; Liam Royce, ISU

Statement of Project Goals

The goal of the project is to characterize the production strains under various operating conditions and to further optimize their performance. The results/data from this project will be used to design omics experiments and to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

The characterization study will assess the effect of genetic manipulations on the performance of the production strains and will provide important data/inputs for improving strains and achieving optimized product production. Specifically, genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the fatty acids produced from Thrust 2 will serve as precursors for the synthesis of α-olefins by Thrust 3.

Fundamental Barriers and Methodologies

1) Carboxylic acids

1A) Effect of host strain and introduced pathways: Host strain and the introduced pathway may have significant effect of the rate and extent of fatty accumulation. In addition, the genetic background of the host strains may also affect the fatty acid chain length distribution. Extensive experimentation is needed to provide insight into the interaction between productivity and production strains.

1B) Effect of acyl-ACP thioesterase and its derivatives: The ability to express functionally active acyl-ACP thioesterase in *E. coli* has shown to have dramatic effect on the accumulation of free fatty acids. Effort will be need to gain a better understanding and develop the ability to design and construct efficient acyl-ACP thioesterase in *E. coli*.

1C) Effect of operating conditions: Operating conditions such as temperature, dissolved oxygen concentration, pH, as well as medium composition often play an important role in process performance. Extensive experimentation will be carried out to quantify these effects.

Achievements

1) Carboxylic acid

Summary: In the past year, we have examined various approaches and screened through a large number of engineered strains to increase the fatty acid production and fatty acid yield. Through this effort, we have developed a highly efficient fatty acid production strain that gives a very high fatty acid **yield close to the maximum theoretical value**. This highly efficient fatty acid production strain is obtained through a combination of developing acyl-ACP thioesterases that have very high biological activities and precise metabolic manipulations of the host strain in the central metabolic pathway and fatty acid biosynthesis pathway.

1A) Characterization of two acyl-ACP thioesterase constructs (mature vs hybrid). The effect of inducer (IPTG) concentration on acyl-ACP thioesterase gene expression, protein quality (soluble vs insoluble) and fatty acid accumulation for two acyl-ACP thioesterase constructs was examined in shake flasks with 40 ml LB broth supplemented with 15 g/l of glucose and varied IPTG concentrations at 30°C (Figs 1A-1 and -2). It is observed that optimum expression of acyl-ACP thioesterase is critical for the strain carrying the mature TE gene, ML103 (pXZM16). The total fatty acid production peaks at around 2.5g/L at 100 µM IPTG and drops to less than 0.15 g/L at higher IPTG concentrations (>300 µM). The TE mRNA concentration peaks at around 250 µM IPTG. Interestingly, the soluble fraction of the TE protein drops sharply for IPTG > 250 µM; the insoluble fraction simultaneously increases significantly also for IPTG > 250 µM. The results suggest the expression of functional acyl-ACP thioesterase is very challenging and requires careful fine-tunings of the expression level to reduce inclusion body formation.

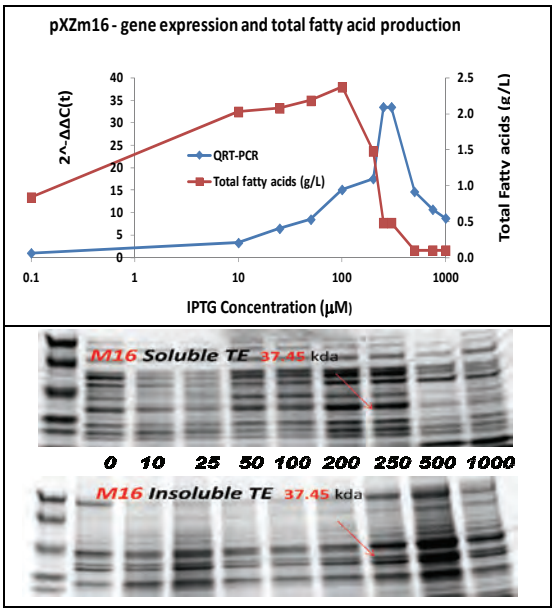


Fig 1A-1. Total fatty acid accumulation, RNA levels, soluble and insoluble proteins of strain 103(pXZm16) as a function of IPTG concentration

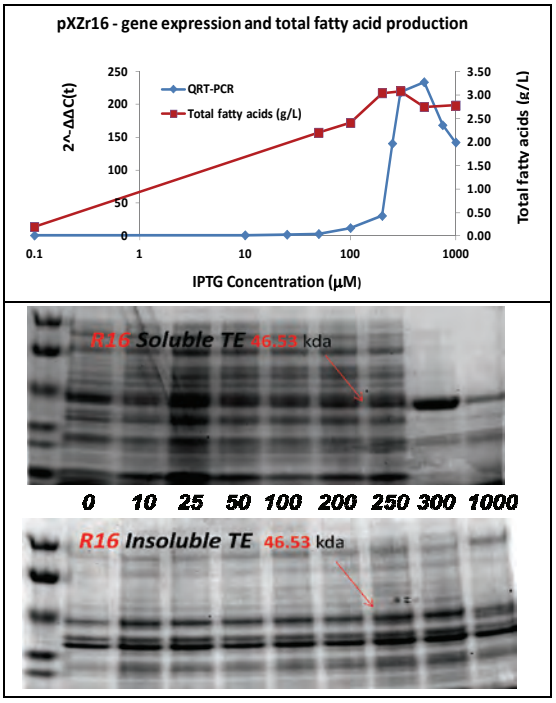


Fig. 1A-2. Total fatty acid accumulation, RNA levels, soluble and insoluble proteins of strain 103(pXZm16) as a function of IPTG concentration

Two approaches were examined to improve the expression of functional acyl-ACP thioesterase: 1) through the construction of a fusion protein to increase protein solubility and 2) deletion of the hydrophobic segment in the N-terminal region of the acyl-ACP thioesterase (based on the results from simulation studies by David Cantu of Thrust 1. A plasmid, pXZR16, carrying a modified m16 thioesterase was constructed and characterized (R16 carries the leading sequence from castor bean). The response of this strain, ML103(pXZR16) is quite different from that of ML103(pXZM16) which carries the mature TE. The added leading sequence from castor bean is able to stabilize the acyl-ACP thioesterase expression in *E. coli*, leading to more stable and highly efficient

production of fatty acids of more than 3 g/L in ML103(pXZR16), a level at least 20% higher than that of the strain ML103(pXZM16). The gene expression levels, as indicated by the $\Delta\Delta C_T$ values, of the ML103(pXZR16) strain are also higher than that of the strain ML103(pXZM16). Furthermore, the strain ML103(pXZR16) has higher soluble TE protein fraction and lower insoluble protein fraction at higher IPTG levels as compared to that of the original strain carrying the mature TE, ML103(pXZM16). The results suggested that the leading sequence of the acyl-ACP thioesterase from castor bean has a stabilization effect which allows the expression of more soluble TE proteins. These observations are also consistent with the simulation results by David Cantu of Peter Reilly's group (Thrust 1) in which they showed that the hydrophobic segment in the N-terminal region in pXZM16 might lead to the formation of insoluble proteins and hence lower acyl-ACP thioesterase activities.

1B) Characterization of three modified acyl-ACP thioesterase constructs. The effect of inducer (IPTG) concentration on fatty acids accumulation for two modified acyl-ACP thioesterase constructs, pXZT16, pXZM16D37 and pXZR16D37 was examined in shake flasks with 40 ml LB broth supplemented with 15 g/l of glucose and varied IPTG concentrations at 30°C. Fig. 1B-1 shows the shake flask results together with those from 1A. The commonly used thio-based fusion protein approach did not improve the fatty acid production. The strain 103(pXZT16) accumulated only about 1.5 g/L of fatty acid at around 250 μM IPTG. The deletion of the hydrophobic segment in the N-terminal region also did not improve fatty acid production. The response to IPTG of strain ML103(pXZM16D37) is very similar to that of ML103(pXZM16), but peaks at a much lower IPTG concentration (Fig. 1B-1). The total fatty acids produced by the strain ML103(pXZR16D37) is lower than that of ML103(pXZR16). The results again suggested the leading sequence from castor bean has the best stabilization effect leading to a higher fatty acid production.

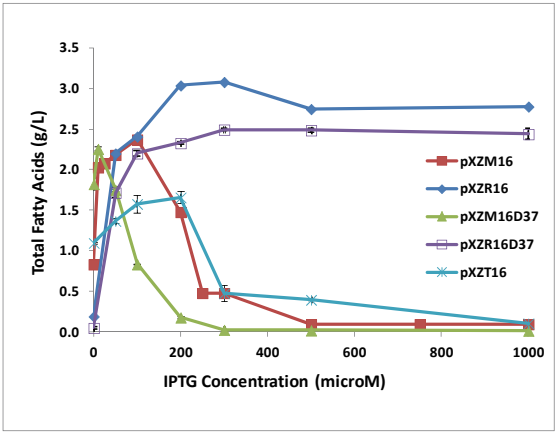


Fig. 1B-1. Effect of induction level on total fatty acid production with the same host strain ML103 carrying different acyl-ACP thioesterases

1C) Effect of central metabolic pathway manipulations on fatty acid production – medium chain fatty acids. The performance of eight strains carrying the same plasmid pXZ18 (containing an acyl-ACP thioesterase) but with different manipulations in the central metabolic pathway was examined in shake flasks with 40 ml LB broth supplemented with 15 g/l of glucose and 1 mM IPTG at 30°C (Fig. 1C-1). Only the 48 hours samples were shown (Data shown were averages of at least 3 replicates). The 24 hours samples showed a similar trend (data not shown). The *sucC*⁻ mutant strain, MLK163(pXZ18), produced the highest total fatty acids at 48 hrs, more than 4 g/L (a very high fatty acids/glucose yield of more than 0.27; this yield value is more than 70% of the maximum theoretical yield). Another TCA cycle mutant strain, MLK193(pXZ18) with *fumCA*, produced fatty acids only slightly higher than that of the reference strain, ML103(pXZ18). The strain with mutations in the glycolysis pathway, MLK191(pXZ18) with *pfkF* gene knock-out, also showed similar fatty acid productivity as the reference strain ML103(pXZ18). Two other mutant strains, MLK190(pXZ18), MLK191(pXZ18) with mutations in the glucose uptake system (*ptsG*), showed inferior performance when compared to that of the reference strain

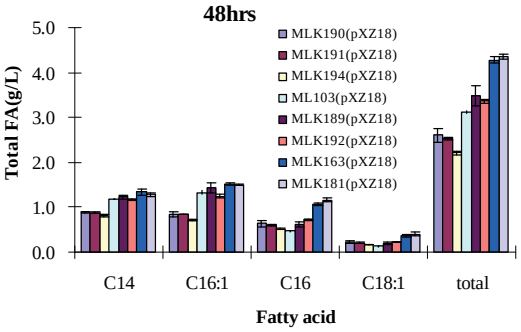


Fig. 1C-1. Total fatty acid production by strains with various genetic manipulations in the central metabolic pathway

(Fig. 1C-1). The fact that various TCA cycle mutant strains showing drastically different performance, with the *sucC* mutant strain out-performs the *fumCA* mutant strain and *gltA* mutant strain (data not shown), demonstrates the complexity of the system involved.

1D) Effect of manipulating gene(s) involved in the fatty acid biosynthesis on fatty acid production. The performance of various strains carrying the same acyl-ACP thioesterase but with different manipulations in the fatty acid synthesis pathway with and without *sucC* mutation was examined in shake flasks with 40 ml LB broth supplemented with 15 g/l of glucose and 1 mM IPTG at 30°C. The results of 14 strains that exhibited various responses are summarized in the table below.

Table 1D-1: Performance of various engineered strains compared with the control strain ML103(pXZ18)

	Strain name	Relevant genotype	% improvement in FA titer*	% improvement in FA /glucose yield*
Reference	ML103(pXZ18)	Δ*fadD*	-	-
Positive effect	MLK211(pXZ18)	Δ*fadD* Δ*fabR*	20	19
	MLK212(pXZ18)	Δ*fadD*Δ*sucC*Δ*fabR*	23	24
	MLK163(pXZ18)	Δ*fadD*, Δ*sucC*	27	29
	ML103(pXZ18 fadR)	Δ*fadD* $fadR^{++}$	34	29
	ML103(pXZ18Z)	Δ*fadD* $fabZ^{++}$	48	48
	MLK225(pXZ18Z)	Δ*fadD* Δ*fadR* $fabZ^{++}$	19	19
⟶	**MLK163(pXZ18Z)**	**Δ*fadD* Δ*sucC* $fabZ^{++}$**	**81**	**81**
Negative effect	ML103(pXZ18A)	Δ*fadD* $fabA^{++}$	-86	-67
	MLK163_18A	Δ*fadD* Δ*sucC* $fabA^{++}$	-35	-19
	MLK225(pXZ18)	Δ*fadD* Δ*fadR*	-18	-19
	MLK227(pXZ18)	Δ*fadD* Δ*fadR* Δ*fabR*	-28	-19
	MLK163(pXZ18 fadR)	Δ*fadD*Δ*sucC* $fadR^{++}$	-52	5

$fabA^{++}$ = overexpression of FabA; $fabZ^{++}$ = overexpression of FabZ;
$fadR^{++}$ = overexpression of FadR; * percentage improvement based on ML103(pXZ18)

Important observations are:

Positive Effect - Several of the manipulations improve fatty acid production, for example:

1) Inactivation of the transcription factor FabR improves fatty acid production and yield for the parent strain (MLK211(pXZ18) vs ML103(pXZ18); however, the same manipulation does not improve the fatty acid productivity of the *sucC* mutant strain (MLK212(pXZ18) vs MLK163(pXZ18)).
2) Overexpression of FabZ improves fatty acid production and yield for both the parent strain and the *sucC* mutant strain (ML103(pXZ18Z) vz ML103(pXZ18) and MLK163(pXZ18Z) vs MLK163(pXZ18)). In fact, a combination of *sucC* inactivation and FabZ overexpression yields the best strain with a very high titer and a very high yield of more than 0.35 g FA/g glucose (which is more than 80% improvement of the base strain ML103(pXZ18)). The yield of more than 0.35 g/g by the MLK163(pXZ18Z) is very close to the maximum theoretical value (The maximum theoretical yield of C14 and C16 straight chain fatty acids are 0.3629 and 0.3561 g/g, respectively).
3) Overexpression of FadR improves fatty acid production and yield for the parent strain (ML103(pXZ18fadR) vz ML103(pXZ18)).
4) Order of improvements: Δ*sucC* $fabZ^{++}$ > $fabZ^{++}$ > $fadR^{++}$ > Δ*sucC* > Δ*sucC*Δ*fabR* > Δ*fabR* or Δ*fadR* $fabZ^{+}$ > Δ*fabR* $fabZ^{+}$

Negative Effect - Several of the manipulations impair fatty acid production, for example:

1) Overexpression of FabA significantly decreases fatty acid production and yield for both the

parent strain and the *sucC* mutant strain (ML103(pXZ18A) vz MLK103(pXZ18) and MLK163(pXZ18A) vs ML163(pXZ18)).

2) Simultaneous deactivation of *fadR* and *fabR* decreases fatty acid production and yield for the parent strain (ML227(pXZ18) vz MLK103(pXZ18)).

The gene expression levels of 14 selected genes surrounding the fatty acid synthesis cycle of 9 selected strains were examined using standard qRT-PCR protocols to shed some lights into the effect of genetic manipulations on the fatty acid synthesis pathway (data not shown).

1E) Effect of TE constructs on short chain fatty acid production.

The performance of the three strains carrying different acyl-ACP thioesterases was examined with 40 ml LB broth supplemented with 15 g/l of glucose and 1 mM IPTG at 30°C (Figs 1E-1). The control strain, K27(pXZmCP) produced less than 0.03 g/L of octanoic acid. The other strains, K27(pXZCP80) and K27(pXZCP88), carrying modified acyl-ACP thioesterases produced much higher octanoic acid, > 0.3 g/L, than that of the control strain K27(pXZmCP) (Fig. 1E-1). The results suggest the important role of acyl-ACP thioesterases in the production of short chain length fatty acid. Higher fatty acid titers can be obtained by improving the biological activity of the thioesterases involved.

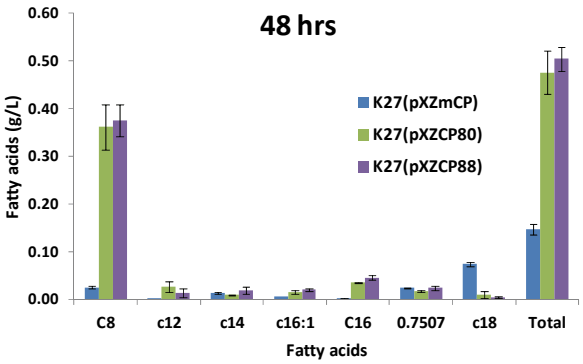


Fig. 1E-1. Fatty acid production by strains carrying different acyl-ACP thioesterases design for octanoic acid production.

1F) Effect of central metabolic pathway manipulations on fatty acid production – shorter chain fatty acids. The performance of six strains with central pathway manipulations carrying the same plasmid pXZCP88 (carrying an acyl-ACP thioesterase designed for octanic acid (C-8) formation) was examined in shake flasks with 40 ml LB broth supplemented with 15 g/l of glucose and 1 mM IPTG at 30°C (Fig. 1F-1). Only the 48 hours samples were shown (Data shown were averages of at least 3 replicates). The 24 hours samples showed a similar trend (data not shown). Only the production of octanoic acid is shown since these strains produced relatively pure octanoic acid. The control strain ML103(pXZCP88) produced less than 0.1 g/L of octanoic acid. All the engineered strains produced more octanoic acid than the control strain, except the *glk* mutant strain MLk189(pXZCP88), see Fig. 1F-1. The two best strains, MLk190(pXZCP88) and MLk191(pXZCP88), produced more than 1.2 g/L of octanoic acid. Note that the strains that performed well for octanoic acid production are quite different from those that performed well for the longer chain length fatty acid (Fig. 1C-1). These results suggest that different host strain manipulations are needed depending on the chain length of the fatty acid to be produced.

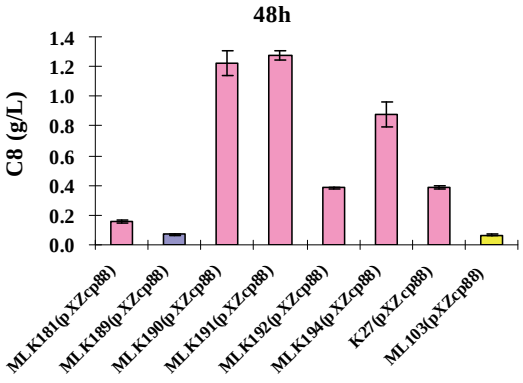
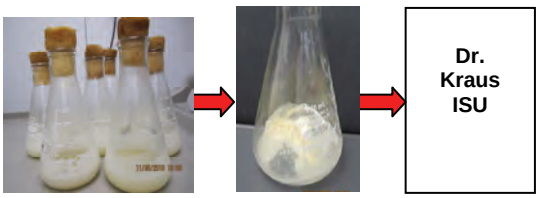


Fig. 1F-1. Octanoic acid production by strains with various genetic manipulations in the central metabolic pathway.

1G) Interactions with Thrust 3: Free fatty acids using the strain ML103(pXZ18) were produced using shake flasks. The fatty acids were prepared (see figure on right as previously described) and the materials were sent to Dr. George Kraus of Thrust 3 at ISU for further study (conversion to alpha-olefin).



1H) Carboxylic Acids Impact Membrane Polarization. Given that our desired products (carboxylic acids) are expected to be partially soluble in the lipid-based cell membrane, it is possible that the membrane fluidity could change in the presence of carboxylic acids. This effect of octanoic acid on *E. coli* MG1655's membrane fluidity was verified through the use of diphenyl, 1,3,6 hexatriene (DPH), a fluorescent probe that aids in measurement of the polarization of the membrane (Fig 1.H-1). Note that membrane fluidity is inversely related to the polarization value. Not only did we find that 30mM exogenous carboxylic acids decrease the membrane polarization by, approximately 30%, the impact is much more severe than ethanol or heating to 42^0C. Note that the measurements were performed in the presence of exogenous octanoic acid. Measurements of membrane polarization during the course of carboxylic acid production are in progress.

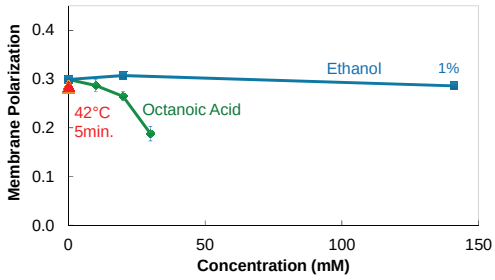


Fig. 1H-1: Octanoic acid has a much stronger impact on membrane polarization (i.e. fluidity) of M1655 than ethanol or heat shock.

While the decrease in polarization correlates with growth inhibition (*data not shown*), it has not been established that this change in polarization is the cause of the growth inhibition. However, it is expected that this decrease in membrane polarization could impact critical cell properties that occur in the membrane, such as the aerobic electron transport chain.

1.I) Carboxylic Acids Impact Membrane Integrity

One of the key functions of the cell membrane is to keep valuable metabolites sequestered inside the cell. Given the observed impact of carboxylic acids on membrane polarization (fluidity), we tested the ability of the *E. coli* MG1655 membrane to retain small molecules in the presence of carboxylic acids. Due to its easy, rapid, measurability, Mg^{2+} was our model metabolite. Fig. 1.I.1 shows the drastic impact of octanoic acid on Mg^{2+} leakage; as with membrane polarization, the impact is much more severe than with the known inhibitors ethanol and heat shock.

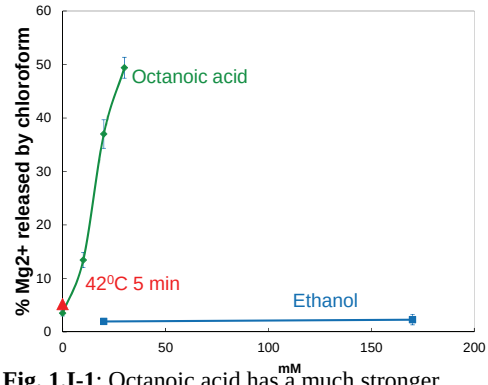


Fig. 1.I-1: Octanoic acid has a much stronger impact on Mg^{2+} leakage of M1655 than ethanol or heat shock.

Though the magnitude of the leakage correlates with the observed growth inhibition (data not shown), it has not been demonstrated that this leakage is the cause of the growth inhibition.

This correlation between the presence of carboxylic acids and Mg^{2+} leakage was also observed during carboxylic acid production by the engineered strain. *E. coli* ML103+pXZ18Z was grown in M9 minimal media with induction by IPTG in temperature, pH, dO_2 and stir-controlled fermentors. During the period when carboxylic acid titers were low, Mg^{2+} leakage was relatively low. Mg^{2+} leakage drastically increased as carboxylic acid titers increased. Note that in the same strain in the absence of plasmid induction, carboxylic acid titers did not exceed 0.1 g/L and leakage values remained below 30% (*data not shown*). This data suggests that the engineered strain is losing valuable metabolites during carboxylic acid production; mitigating this leakage could enable increased carboxylic acid production. Preliminary results have shown that altering lipid A content

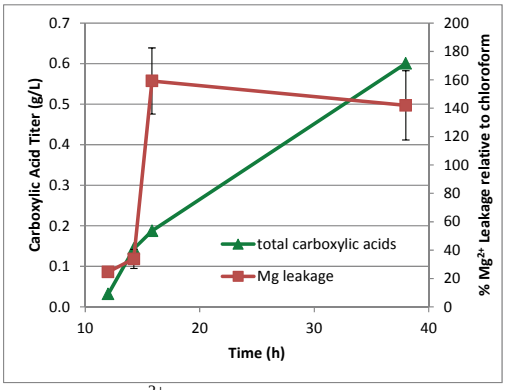


Fig. 1.I-2: Mg^{2+} leakage increases with carboxylic acid titer for ML103+pXZ18Z during growth in M9 glucose with IPTG.

alters leakage in the presence of octanoic acid (*data not shown*). Alternatively, it is also possible that the leakage enables efflux of the product and is not detrimental to strain performance.

2. Methyl ketone

Effect of dissolved oxygen concentration on methyl ketone synthesis The differences in methyl ketone yield and concentration observed under different aerobicity levels in shake flasks (reported last year) led to the study of the effect of dissolved oxygen concentration on methyl ketone synthesis in order to find the best culture conditions in a controlled bioreactor vessel. In order to quantify the specific dissolved oxygen percentage (DO%) that optimized yield and titer, and to get a better grasp on how these metrics change with specific changes in DO, several bioreactor fermentations were conducted with different DO set-points. The best performing strain from the shake flask experiments (MG1655 Δ*adhE* Δ*ldhA* Δ*poxB* Δ*pta* pTrcHis2A-shmks2-mks1: detailed in last year's report) was cultured at different dissolved oxygen values ranging between 2% and 20%. The strain was cultivated in a 500 mL bioreactor vessel in which pH and dissolved oxygen were tightly controlled. MOPS minimal media was supplemented with 20g/L glucose. The pH was set at 7, temperature at 37°C, and cultures were harvested at 12h. The synthesis of methyl ketones by these cultures is shown in Fig. 2-A.

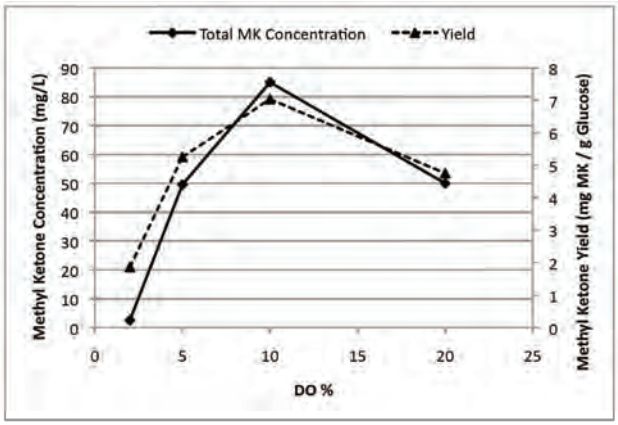


Fig. 2-A. Effect of DO%

The bioreactor cultures exhibited a marked increase in both the concentration and yield of methyl ketones over those obtained from shake flasks. Several parameters that changed from the shake flasks to the bioreactors may be responsible for this drastic improvement of methyl ketone production. The first is more consistent oxygen availability. A study of dissolved oxygen content over the course of *E. coli* and yeast fermentations in 250 mL shake flasks with respect to culture volume and agitation, showed high variability of DO during the fermentations (Tolosa et al., Biotechnol. Bioeng. 80, 594-597, 2002). Specifically, the authors encountered the onset of oxygen limitation (DO measured at ~0%) for both 50 mL and 75 mL culture volumes and at 150, 250, and 350 RPM shaker speeds. During late exponential and early stationary phase, the dissolved oxygen content rapidly increased back to almost 100% saturation. Conversely, DO in the reactor was monitored and kept steady at specified set-points above 0% saturation. In other words, the increased availability of oxygen in the media, however low the saturation level, may have greatly improved the production of methyl ketones. Secondly, there may have been losses of methyl ketones through evaporation in the shake flasks, as methyl ketones have been detected in the headspace of methyl ketone-producing *E. coli* cultures (Fridman et al., Plant Cell. 17, 1252-1267, 2005). In the bioreactor setup, a condenser is placed between the headspace and the headspace outlet to minimize losses of volatile compounds. Thirdly, it is widely known that there are optimal ranges of pH for maximum activity and stability of enzymes. Shake flask cultures, even when supplemented with calcium carbonate, exhibited decreases in pH from 7 to 5.0-6.0 in some cases. Due to the more controlled environment in the bioreactor, the pH was

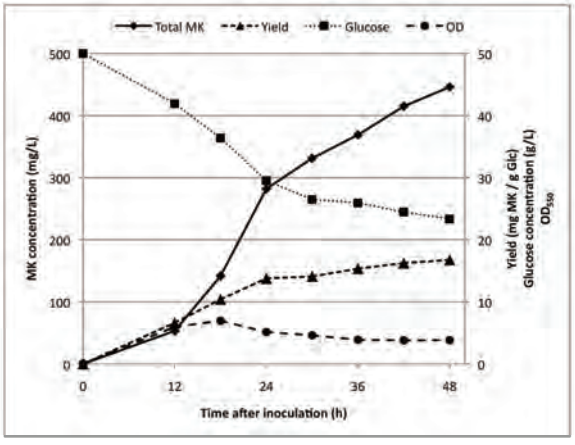


Fig. 2-B. Profile of best performer at DO 10%

maintained constant at 7.0 throughout cultivation. This may have had a positive effect on the activity of SHMKS2 and MKS1.
According to strain performance at 12 hours, the maximum yield and concentration values are attained when the culture conditions are set at DO 10% (Fig. 2-**A**). To further characterize the best performer strain under these optimal growth conditions, it was cultivated in the bioreactor using a DO setting of 10% and glucose supplementation of 50 g/L. The results of this fermentation (Fig. 2-B) show methyl ketone titers approaching 450 mg/L by 48 hours, and a yield leveling off at 17 mg MK / g Glucose. Both metrics are the highest recorded for any of the constructed strains or any engineered microorganism reported thus far. Growth entered late exponential to stationary phase after 12 hours, at which point the rate of glucose consumption steadily decreased, as expected. If total glucose consumption is increased, this final strain could achieve even higher methyl ketone titers.

Other Relevant Work

n/a

Plans for the Next Year

1 Carboxylic acids

The plans for the next five years are very similar to that of project 1A. Specifically, the focus will on producing shorter chain carboxylic acids with high purity and high titer. We will be designing and performing characterization experiments to study the fatty acid producing strains developed in the *E. coli* strain construction project (1A). Furthermore, we will study the effect of various key operating conditions on strain performance.

Expected Milestones and Deliverables

The deliverables for the coming year will be quantified assessment of the performance of the *E. coli* strains plasmids and acyl-ACP thioesterase developed in Project 1A and 2A under different culture conditions. In addition, results from these characterization studies will guide the design and construction of second-generation fatty acid production systems with improved performance.

Member Company Benefits

The knowledge and constructs (plasmids and strains) being developed in this project will be useful to member companies. The knowledge leading to the possibility of producing medium to short chain carboxylic acid at high purity and high titer will have many other potential applications.

Commercialization / Technology Transfer

We are actively engaging with Center members and other companies, including start-ups, for potential licensing and commercialization of the carboxylic acid production technologies (five invention disclosures, 1 PCT application and 1 provisional application).

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.2B -** Strain Characterization and Optimization in *S. cerevisiae*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Nancy A. Da Silva	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Faculty: Nancy Da Silva (UCI), Suzanne Sandmeyer (UCI), Laura Jarboe (ISU)

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Undergraduate Students: Christina Tran (UCI), Aidan Gilchrest (REU student; University of Massachusetts)

Statement of Project Goals

The goals of the work are to characterize the *Saccharomyces cerevisiae* strains under various operating conditions and to further optimize their performance for high level synthesis of carboxylic acids and pyrones.

Project's Role in Center's Strategic Plan

The goal is to characterize microbial strains for the production of two test bed chemicals, carboxylic acids and pyrones. These two test beds provide opportunities to integrate all three research thrusts. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2, short chain fatty acids and pyrones will serve as precursors for the synthesis of α-olefins, dienes, and other compounds by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

Strain characterization requires methods to analyze both the amounts and identities of the fatty acids (FAs) and related products. Further optimization requires methods to rapidly assess the effects of genetic and environmental changes. We are thus developing the needed product assays and reporter gene approaches for strain assessment. To predict strategies for increasing the performance of the strains, we will interact closely with the Omics (Project 3B), Flux Analysis (Project 4B), and Bioinformatics (Project 5B) researchers.

Achievements

During the first three years of the Center, our efforts focused on strain construction (Project 1B) and initial characterization work including the development of required methods and strategies. During

the fourth year, we have made significant progress on the characterization of our strains. We have (1) determined the effects of growth-limiting factors on fatty acid production in the oleaginous yeast *Yarrowia lypolytica*, (2) evaluated the heterologous FAS strains for activity and fatty acid synthesis, including systems for short chain fatty acid (FA) synthesis using TEs from Thrust 1, (3) evaluated strains engineered for increased fatty acid synthesis, (4) evaluated strains for the high-level synthesis of pyrones.

(1) Determination of conditions that limit growth for carbon and for nitrogen and effects on fatty acid (FA) production

Y. lypolytica was tested for growth on pentose (xylose), acetate, lactate, glycerol, and oleate carbon sources. It did not grow on xylose but grew robustly on other carbon sources with minimal supplements (yeast nitrogen base without ammonium sulfate). Growth of *Y. lypolytica* was monitored under conditions where carbon or nitrogen become limiting. The same samples were also used in triplicate to extract fatty acids, followed by methylation. Samples were spiked with reference C19 and GC-MS analysis was performed at the ISU Metabolomics Core Facility. FA recovery was assumed to be similar for endogenous FA and exogenous C19 standard and FA production is expressed normalized to the C19 standard. Nitrogen limitation in replete carbon is reported to trigger FA accumulation. A second set of experiments using *Y. lypolytica* determined limiting nitrogen was achieved in 0.125% ammonium sulfate as compared to 0.5% ammonium sulfate (no amino acids) in 0.11 M glucose, 0.2 M potassium acetate, and 0.22 M glycerol containing medium. Growth curves showed similar doubling times (glucose, 2.54 h; glycerol, 1.84 h; and acetate, 2.93 h). In a separate experiment, triplicates of four time points starting with the onset of nitrogen limitation into late stationary in each carbon source were analyzed for FA accumulation.

Several preliminary conclusions could be drawn from these experiments. First, grown under glucose limitation, *Y. lypolytica* produces significantly more FA than *S. cerevisiae* (Figure 1). Second, cells grew well and produced similar amounts of FA on acetate and glycerol, both of which are generated as waste products and could be important biosustainable sources of carbon for FA production. Third, although our experiments did not measure *Y. lypolytica* FA production prior to nitrogen limitation on the different carbon sources, comparison of different experiments based on the C19 standard normalization, did not show the anticipated increase in FA production upon nitrogen limitation. These experiments will be repeated in order to verify the differences with *S. cerevisiae* and to retest the levels of *Y. lypolytica* FA prior to and subsequent to nitrogen limitation.

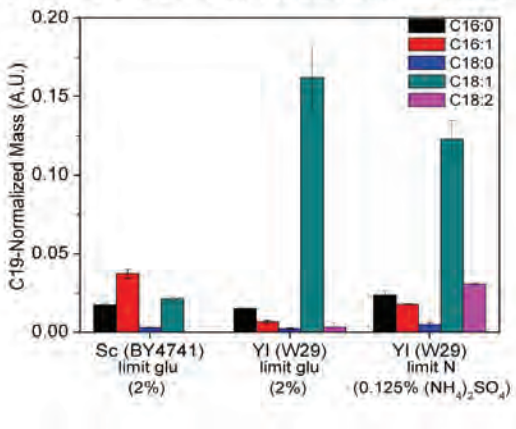

Figure 1: Comparison of Fatty Acid Synthesis in *S. cerevisiae* (BY4741) and *Y. lypolytica* (W29) entering nutrient limitation.

(2) Characterization of heterologous FAS strains for fatty acid synthesis in *S. cerevisiae*

The activity of the mammalian FAS (mFAS) system in *S. cerevisiae* was previously confirmed via complementation of a yeast *FAS2* knockout and growth of the yeast in the absence of exogenous fatty acid supplements. To confirm activity of the *E. coli* FAS system, the nine enzymes were synthesized in yeast and his-tag purified. An *in vitro* ADIFAB assay demonstrated fatty acid synthesis, confirming activity of all 9 enzymes (Figure 2). Integration of the *E. coli* genes into a single strain is underway using an optimized integration method (Project 1B). When complete, *in vivo* assays will be undertaken and complementation of a yeast *FAS2* knockout evaluated.

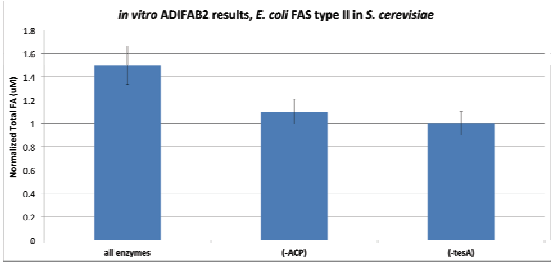

Figure 2: *In vitro* ADIFAB2 assay confirming activity of the *E.coli* FAS type II expressed in yeast.

For the synthesis of short chain fatty acids, we have combined novel thioesterases from Thrust I and two TEs from the literature with our new heterologous yeast systems. The activity of the TEs produced in yeast were confirmed using *in vitro* assays (with highest activity seen at shorter carbon lengths for the enzymes assayed). The TEs with the highest activity have been transformed into the yeast strain carrying mFAS (with or without the TE domain). A new instrument, the GC-GC-MS is being used for short chain fatty acid detection. The GC-GC-MS has the ability to separate analytes by the sequential separation through two tandemly arranged gas chromatography columns. This is beneficial in eliminating long chain fatty acids for an increased sensitivity to shorter chain fatty acids. An initial trial run utilizing GC-GC-MS was conducted. Our mutant mFAS expressed with novel short chain TEs gave significantly more C_{12} and C_{10} production relative to our control; the results with TEII are shown in Figure 3. Further runs are underway to confirm these findings. In addition, the sample preparation method will be changed so that C_6 and C_8 levels can be detected.

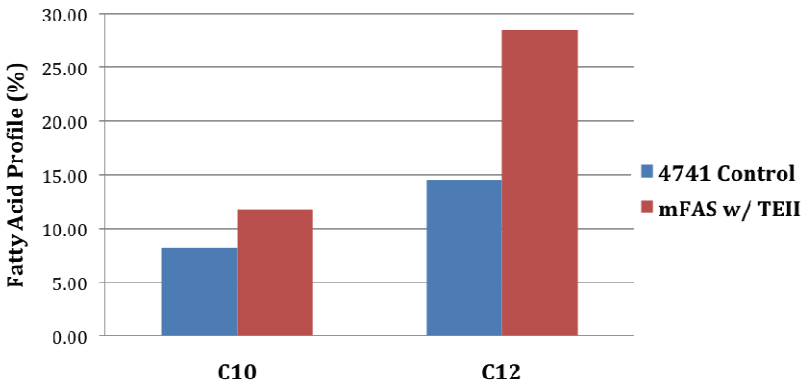

Figure 3: Short chain fatty acid (C_{10}, C_{12}) profile of strains using the GC-GC-MS.

(3) Evaluation of strains engineered for increased fatty acid synthesis

To engineer strains for increased production of fatty acids and related compounds, we have knocked out specific regulatory and pathway genes (e.g., for Opi1 and Snf2, for β-oxidation enzymes) and upregulated genes for the synthesis of important precursors (e.g., acetyl-CoA, malonyl-CoA) (Project 1B). One approach to increase the pool of acetyl-CoA has utilized a strategy from the oleaginous yeasts known to accumulate fatty acids.

Another major focus has been the control and down-regulation of the native yeast fatty acid synthase. The native FAS is the primary consumer of both acetyl-CoA and malonyl-CoA. The overall strategy is to utilize the native FAS for initial growth, then down-regulate this FAS so that the acetyl-CoA and malonyl-CoA pools are available to the heterologous FAS for short chain fatty acid synthesis.

To regulate native FAS expression, we utilized a *FAS2* knockout strain carrying a *FAS2* plasmid that can harbor various promoters, controlling expression time and strength. The native *FAS2* promoter and *PGK1* promoter under a 2μ or CEN/ARS replication origin have been analyzed (Figure 4). These findings demonstrate that the native FAS levels can be controlled by independent expression of the *FAS2* gene on an autonomous plasmid with growth rates comparable to the wild-type. Current experiments are assessing various constitutive and inducible promoters for native FAS synthesis, and *in vivo* fatty acid production will be determined in early exponential, late exponential and stationary phases with the GC-MS.

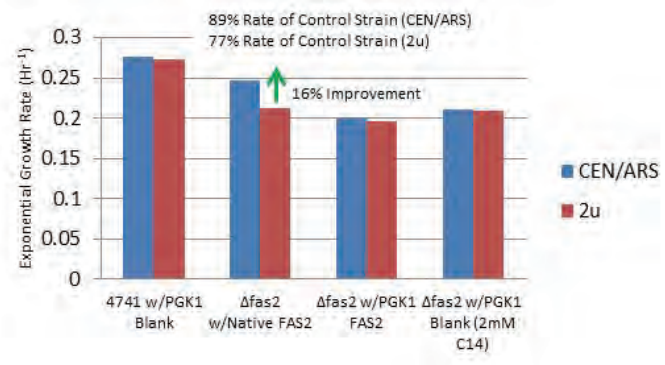

Figure 4: Exponential growth rate comparison of complemented FAS2 knockout strain using 2μ and CEN/ARS origin plasmids.

(4) Evaluation of strains engineered for the synthesis of pyrones

To increase pyrone synthesis, several strategies have been pursued including the assessment of new enzyme variants (developed by the Noel lab, Thrust 1), preventing proteolysis of the synthase, increasing 2-PS expression levels (via copy number and promoter choice), and increasing precursor availability. The triacetic acid lactone (TAL) produced is measured in the culture medium using HPLC.

Six 2-PS mutants from the Noel lab were cloned into pXP vectors and TAL levels up to 50% higher were observed. Additional mutants, including combinations of the more promising ones, are currently being screened. Preventing proteolysis using protease deficient yeast strains was key to increasing pyrone levels (by nearly 3-fold). The results shown in Figure 5 illustrate a series of TAL levels for these strains and the initial group of 2-PS variants evaluated.

In addition, strains are being engineered for higher TAL synthesis. Precursor availability was improved by identifying bottlenecks via computational modeling. Use of OptKnock in the COBRA 2.0 Toolkit indicated that strains inhibited in both fatty acid synthesis and pyruvate carboxylase activity should increase TAL levels (Project 1B). Deletion of either *pyc1* gene, or adding cerulenin to inhibit fatty acid synthesis, resulted in up to 2-fold higher TAL productivities (Figure 6). Current efforts include the down-regulation of fatty acid synthesis to improve pyrone production (in coordination with the efforts for improving short chain fatty acid synthesis described above).

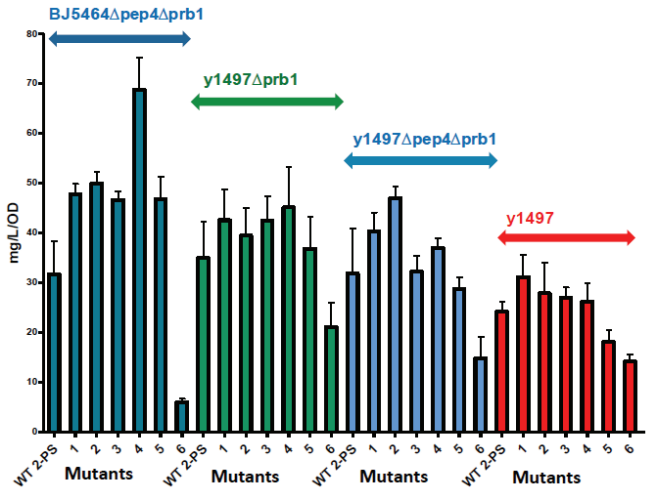

Figure 5: Pyrone levels in protease deficient strains comparing 2-PS mutants to the wildtype enzyme.

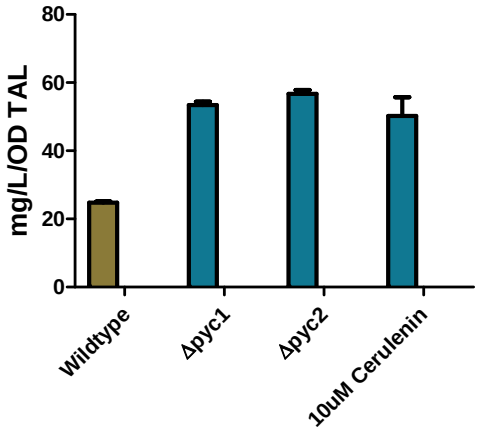

Figure 6: Pyrone levels in engineered strains for increased precursor pools as identified by OptKnock.

To date these manipulations have led to a 15-fold increase in TAL titer and a 30-fold increase in TAL yield (g TAL/g glucose). We are currently producing greater than 1 g/L TAL, and have transferred our culture broth to Thrust 3 for their catalysis work. Ongoing work focuses on combining the most promising strategies, assessing alternate expression strategies, and increasing precursor availability. We have also made significant progress in achieving similar yields in minimal yeast medium, critical for the flux experiments and more relevant for industrial applications.

Other Relevant Work

Similar experimental work is being conducted within CBiRC using *E. coli* as the model microbial system. In combination, the research will evaluate three promising microbial systems for the synthesis of the precursor compounds required for the Center's goals. Other methodologies utilized within CBiRC (e.g., DNA microoarrays, proteomics, and flux analysis) will also provide key information to guide future strain development and characterization.

Plans for the Next Year

Characterize and optimize strains for increased titer and yield of short chain fatty acids and pyrones:

Characterize the strains developed in Project 1B: Measure fatty acid and pyrone levels; evaluate

removal of pathway bottlenecks.

Combine information from Omics, Flux, and Bioinformatics groups with targeted characterization studies to guide further strain development and optimization.

Characterize strains expressing new enzymes from Thrust 1

Expected Milestones and Deliverables

Tools for characterization of strains producing carboxylic acids and pyrones

Determination of FAS/TE combination for synthesis of fatty acids of specified chain length

Identification of key bottlenecks in the synthesis of fatty acids and polyketides

Member Company Benefits

The benefits for the Center's industry members are the characterization and further optimization of strains for the high-level synthesis of carboxylic acids, pyrones, and other desired compounds. The methods developed and the integration with Omics, Flux Analysis, and Bioinformatics efforts will demonstrate the effectiveness of the metabolic engineering optimization cycle.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.3A -** Omics Experiments in *E. coli*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ramon Gonzalez	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Ramon Gonzalez, Rice University
Other Faculty: Laura Jarboe and Julie Dickerson, Iowa State University
Graduate Students: Maria Rodriguez-Moya, Rice University; Liam Royce, Jesse Walsh, and Erin Boggess, Iowa State University

Statement of Project Goals

This project aims to use functional genomics tools to: i) identify the metabolic response of *E. coli* to inhibitory concentrations of short chain fatty acids (SCFA) and pyrones and ii) assess the metabolic changes resulting from the engineering of pathways for the production of SCFA and pyrones in *E. coli*. System-wide characterization of gene and protein expression will be performed by DNA microarrays and 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) combined with Mass Spectroscopy. The outcomes of both objectives will support the engineering of strains able to produce and tolerate high levels of fatty acids and pyrones.

Project's Role in Center's Strategic Plan

The results from this project will directly contribute to both test beds proposed in the Center's strategic plan, namely the production of carboxylic acids and dienes. The functional genomic analysis of strains producing specific products in each of these test beds will contribute to the elucidation of the underlying mechanisms mediating their metabolic performance. These results, in turn, will guide engineering efforts to construct high-producing and high-tolerant strains. The establishment of this systems biology based approach would be of great assistance in the design of other biocatalysts.

Fundamental Barriers and Methodologies

This project could be limited, in general, by the ability to integrate functional genomics approaches into the traditional strain development/metabolic engineering cycle.
Data analysis and interpretation of combined functional genomics studies could also be a barrier.
New approaches and techniques currently under development in the "Bioinformatics" projects will be of tremendous help in overcoming the above barriers.

Achievements

Proteomic studies in rich medium

A Proteomic study had been conducted to analyze the effects of octanoic acid on *E. coli* MG1655.

Growth of the MG1655 wild-type under octanic acid stress (15mM $C_{8:0}$) was compared to growth in the absence of the acid using 2D-gel electrophoresis. Several differentially expressed proteins had been previously identified via MS analysis, while additional differentially expressed spots have been recently identified by gel-matching methods using *E. coli* master gels from online databases, mainly ExPASy, and proteins identified via MS as landmarks (Figure 1, Table 1).

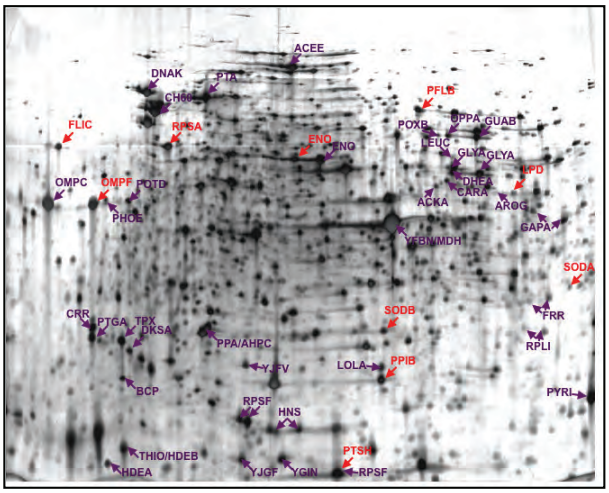

Figure 1. 2D-gel map incorporating differentially expressed proteins identified via MS analysis (red) and using gel-matching methods with master gels from online databases and proteins identified via MS as landmarks (purple).

Table 1. Differentially expressed proteins in *E. coli* MG1655 in response octanoic acid stress (15mM).

Protein Name (*gene*)	Differential Expression (fold)	Protein Function
Hpr (*ptsH*)	(-) 6.5	Non-specific sugar transport, PTS system
Mn-Superoxide Dismutase (*sodA*)	(-) 6.4	Response to oxidative stress
Fe-Superoxide Dismutase (*sodB*)	(-) 6.0	Response to oxidative stress
Rotamase B (*ppiB*)	(-) 5.7	Protein folding
Outer Membrane Porin F (*ompF*)	(-) 2.0	Transport of medium and long-chain FAs
Pyruvate Oxidase Monomer (*poxB*)*	(-) 2.8	Pyruvate + a ubiquinone + H_2O → CO_2 + a ubiquinol + acetate
Flagellin (*fliC*)	(+) 3.2	Flagellar structure and motility
Ribosomal Protein S1 (*rpsA*)	(+) 4.6	Protein synthesis (mRNA translation)
Enolase (*eno*)*	(+) 4.5/(+) 2.8	2-Phosphoglycerate ↔ Phosphoenolpyruvate + H_2O + H^+
Pyruvate Formate Lyase (*pflB*)	(+) 6.6	Formate + Acetyl-CoA ↔ Pyruvate + Coenzyme A
Dihydrolipoamide Dehydrogenase (*lpd*)	(+) 2.2	Subunit of pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, and glycine cleavage system
Thioredoxin 1(*trxA*)*	(+) 7.8	Disulfide bond reductant-oxidant
Unidentified	(-) 2.3	
Unidentified	(-) 5.8	
Unidentified	(+) 4.0	
Unidentified	(-) 4.1	

(+) up-regulated / (-) down-regulated under octanoic acid stress. Proteins with (*) were identified using gel matching method. Unidentified spots will be identified with further analysis via MS and as additional information for gel matching is acquired.

After organizing the identified proteins into three main categories, ((1) stress response, (2) transport and structural and (3) metabolic functions), several hypotheses have been developed. Some examples are presented in Figures 2 and 3.

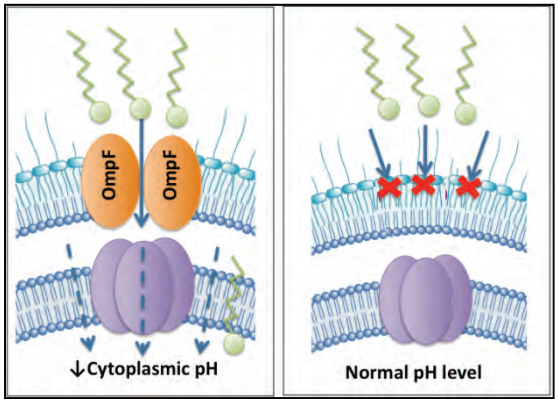

Figure 2. Proposed model of short-chain fatty acid transport across the outer cell membrane via OmpF. OmpF is hypothesized to mediate transport of octanoic acid across the outer membrane, disrupting intracellular pH and oxidative balance (left). Decreased levels of OmpF should limit transport of octanoic acid (right).

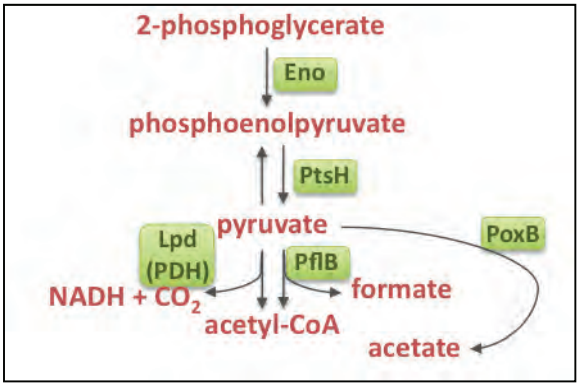

Figure 3. Several differentially expressed proteins are involved in metabolic functions around the phosphoenolpyruvate-pyruvate node. This important node in central carbon metabolism is clearly affected by the exposure to octanoic acid. Analysis of the distribution of fluxes and metabolites at this node could reveal key information about short-chain fatty acid toxicity mechanisms in the cells.

Role of differentially expressed proteins

As an initial approach to assess the role of the differentially expressed proteins related to tolerance of *E. coli* to fatty acids, we analyzed the effect of overexpression and knockout of individual genes. Knockout strains that exhibit a significant difference in growth with respect to the wild-type could suggest a strong influence of that particular gene/protein on tolerance to fatty acids. Mutants from the Keio collection were used to assess these roles. (Knock-outs for *eno* and *rpsA* could not be obtained, since these are essential genes for *E. coli* growth). The ratio of maximum OD was calculated for each mutant strain in order to determine if the mutation is beneficial or detrimental (Figure 4).

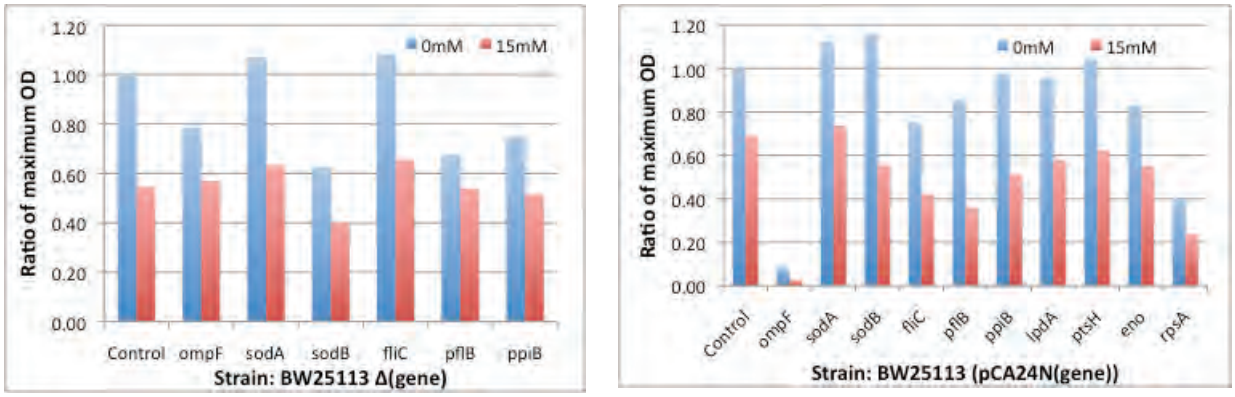

Figure 4. The ratio of maximum growth for each knock-out (left) and over-expression (right) mutant was calculated as [(Max OD of Mutant in 0 or 15mM)/(Max OD of Control in 0mM)]. The control used for the knock-out mutants was wild-type BW25113 and for the over-expression mutants, BW25113 pCA24N (blank vector).

Of particular interest in this analysis is the dramatic effect that the deletion of *ompF* has over cell growth. This outer membrane porin has been proposed as a participant in transport of longer-chain fatty acids across the cell membrane, and has been reported as having an effect on multidrug tolerance (Antimicrobial Agents and Chemotherapy, 2009, 53:4944-4948). Additional studies on the effects of different levels of expression of this and other outer membrane porins could provide valuable information about fatty acid transport across the membrane and about their toxicity mechanisms. The modifications of superoxide dismutases (SodA and SodB) also show interesting differences in growth. The study of the deletion and over-expression of *trxA*, (Thioredoxin 1, which was recently found to be differentially expressed) and measurements of reactive oxygen species (ROS) levels in the cell could provide additional information about the oxidative state of the cells under fatty acid stress. In addition, combined modifications of genes involved in central carbon metabolism, particularly the pyruvate node, could result in improved traits for octanoic acid tolerance in *E. coli*. As can be observed from Figure 4, it is difficult to select individual genes with significant advantages over the control strain. This suggests that, even though some of the mutations (e.g., deletion of *ompF* and *fliC*) give some advantage over the control strain, these genes are not essential, but they might be part of a larger toxicity response mechanism.

Network Component Analysis of Transcriptome Data

As described in previous reports, we have performed transcriptome analysis of *E. coli* MG1655 during challenge with 10mM octanoic acid (C8) during growth in MOPS 2% glucose minimal media, 37^0C, pH 7.0. This dose of C8 is sufficient to decrease the specific growth rate by approximately 10%. Network Component Analysis of transcriptome data (Yao Fu, Dickerson group) identified several regulators with significantly altered activity. This report focuses on two perturbed regulators identified in our analysis, Lrp and GadE.

Leucine-responsive regulatory protein Lrp is a global regulator. Binding of this regulator can influence whether it binds to (and regulates) certain target genes. It has previously been shown that binding of Lrp to short-chain carboxylic acids can lead to replication of the leucine-binding effect (Noriko 2009). Thus, our identification of perturbed regulatory activity of Lrp in the presence of C8 is expected and validates our analytical method. To test the importance of Lrp in the octanoic acid response, we performed growth analysis of both an *lrp* knockout and a strain overexpressing

lrp via the pCA expression vector; neither strain had significantly altered C8 sensitivity relative to the wild-type (data not shown). Thus, while Lrp's regulatory activity may be significantly perturbed in the presence of C8, this interaction does not impact the growth sensitivity of *E. coli* to this inhibitor.

The other key regulator identified in our analysis is GadE, where GadE senses and manages the response to acid stress and membrane damage. This finding led to our proposition that C8 may (a) acidify the cell interior and (b) damage the cell membrane. These propositions were explained in detail in last year's report. Here we provide supporting evidence for each of these.

The transcriptome data shows that the cells are activating (i.e. trying to use) the glutamate-dependent acid resistance system. This system, along with the arginine-dependent acid resistance system, converts these amino acids to GABA and agmatine, respectively, by the addition of a proton. In this manner, these compounds act as proton sinks. We have supplied the cells with either 10mM glutamate or 10mM arginine while being challenged with 10mM C8. However, this supplementation has no significant impact on either C8 tolerance or the intracellular pH. Future efforts involve measurement of GABA and agmatine to determine if these acid resistance pathways are functioning.

Thus, this data shows that one of the effects of C8 challenge is acidification of the cells, despite efforts to control the external pH. We are currently working on cloning of the proton-buffering peptide (*Applied Biochemistry and Biotechnology* 2006, 134:15-26). This short peptide contains basic amino acids that allow excess protons to bind to the protein and thus buffer intracellular pH. Efforts are also underway to determine if the carboxylic-acid producing strain shows this same drop in intracellular pH. A roadblock to these measurements is the fact that the pH-reporter system and thioesterase are currently encoded on incompatible plasmids; this is currently being addressed.

The other implication of GadE activation is possible damage to the cell membrane. We have measured membrane fluidity and membrane integrity (describe in "T2.1A. Strain Construction and Optimization in *E. coli*" report). We have observed that C8 challenge significantly impacts both the membrane fluidity, as evidenced by polarization measurements with a fluorescent probe, and membrane integrity, as evidenced by leakage of Mg^{2+}. This Mg^{2+} leakage was observed during carboxylic acid production by the engineered strain; efforts are underway to assess the impact of carboxylic acid production on membrane fluidity.

Measurements of intracellular pH

Using a GFP reporter system, we have measured the intracellular pH in the presence of C8 (Figure 5). These results show that even though we are constraining the extracellular pH to neutral, as would happen in an industrial fermentation, the intracellular pH in the presence of C8 reaches levels as low as 6.3. This effect differs from a strong acid such as HCl, where compensation for the acidification of the bulk solution results in a neutral intracellular pH. We believe it is the fact that the carboxlic acids are partially soluble in the cell membrane that leads to this acidification effect. The protonated acid can diffuse through the membrane and then dissociate in the cytoplasm. This acidification could burden the cells by (a) inhibiting the activity of crucial pH-sensitive enzymes and (b) result in the loss of ATP in an effort to remove the excess protons.

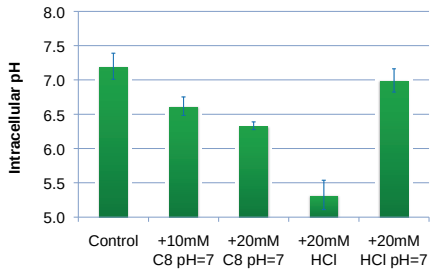

Figure 5. Intracellular pH measurements for *E. coli* MG1655.

Other Relevant Work

Not applicable.

Plans for the Next Year

Transcriptomic and proteomic studies will be performed to obtain more information about key genes and proteins that are responsible for fatty acid tolerance in *E. coli*, including the analysis of a C8-tolerant evolved strain.

Strains will be constructed according to the gene deletions and over-expressions that are selected as most favorable from the enzyme activity and metabolic assays that will be performed.

The role of differentially expressed gene/proteins in the tolerance of *E. coli* to fatty acids will be established using genetic and biochemical approaches.

Gene and protein expression profiling will be obtained for *E. coli* strains exposed to pyrones and the role of differentially expressed genes/proteins will be established.

Integration of gene and protein expression data with metabolic flux data in strains engineered to produce carboxylic acids and pyrones to guide further metabolic engineering efforts.

Expected Milestones and Deliverables

For next year, we expect to:
1. Study variations in expression of outer membrane porins, measure enzyme activities and ROS (reactive oxygen species) levels and other biochemical studies to elucidate the role of proteins postulated to be involved in tolerance to fatty acids.
2.Study the effect of over-expressing and knocking out additional differentially expressed genes/proteins.
3.Clone proton-buffering peptide to buffer intracellular pH.
4. Use high-throughput method to quickly transform and screen for carboxylic acid tolerance.
5. Decrease expression of genes involved in membrane lipid biosynthesis (*lpcA* and *lpxA*) in an attempt to decrease growth sensitivity and Mg^{2+} leakage.

Member Company Benefits

The understanding of the response of *E. coli* to inhibitory concentrations of SCFAs and its harnessing to obtain strains that are tolerant to high concentrations of fatty acids is expected to generate significant intellectual property, which in turn will benefit member companies.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.3B** – Omics Experiments in *S. cerevisiae*

Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Laura R. Jarboe	**02/17/2012**	**03/01/2011 to 02/29/2012**

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Postdoctoral Researchers: Tarek Najdi, Fang Fang, University of California, Irvine

Graduate Students: Ping Liu, Jon Hurst, Iowa State University;

Undergraduate Students: Kimberly Booe, Iowa State University; Charles Henkle, Lousiana State University (REU)

Statement of Project Goals

The goals of this project are two-fold: (1) analyze strains that have increased production of the target compound, in order to formulate strategies for additional strain improvement and (2) analyze our biocatalyst during challenge with an inhibitory concentration of the target compound, in order to engineer the strain or growth condition to alleviate this inhibition. In the work described here, short-chain carboxylic acids are the target product.

Project's Role in Center's Strategic Plan

This project serves the central CBiRC Strategic Plan by contributing to the carboxylic acid test bed and will lead to the development of a standard method for strain optimization and characterization for future test beds. Specifically, this project will aid in both the understanding of the fatty-acid biocatalytic machinery and in the design of efficient biocatalyst systems and contributes to the T2 and T3 critical milestones.

Fundamental Barriers and Methodologies

This project is limited, relative to the *E. coli* project, by the decreased availability of pathway and annotation data of *S. cerevisiae*.

The goal to perform transcriptome analysis and flux analysis in parallel leads to increased stringency for experimental design, given the experimental constraints for flux analysis.

Simultaneous analysis of transcriptome and fluxome data will present technical barriers in terms of data storage and visualization.

Simultaneous interpretation of transcriptome and fluxome data is relatively new to this area.

Achievements

(1) Analyze strains with increased production of the target compound

While construction of our carboxylic acid-producing *S. cerevisiae* continues, omics analysis has focused on the oleaginous yeast *Yarrowia lipolytica*. Note that *Y. lipolytica* is able to accumulate lipids at up to 50% of their dry mass. The goal of this analysis is to understand how lipid metabolism differs between this species with its desirable metabolic properties and our own biocatalyst. The goal of our analysis is to inspire metabolic engineering strategies for *S. cerevisiae* enabling increased carboxylic acid production. Genome sequence analysis of *Y. lipolytica* W29 by our group is described in the yeast bioinformatics report.

Y. lipolytica was grown in SD complete media and RNA was extracted from triplicate samples after 9, 17, 32 and 52 h of growth corresponding to the log, late log, stationary and late stationary growth phases, respectively. RNAs were processed into cDNA libraries and sequenced. Gene expression was calculated by normalizing the number of reads at each gene to the gene length and to the total number of mapped gene reads per sample (RPKM: Reads Per Kilobase of gene per Million mapped reads). Cyber-T software was used to perform statistical analysis of gene expression. The cut-off for Bayes P-values, posterior probability of differential gene expression (PPDE) and fold difference values were 0.001, 0.999 and 1.3, respectively. Spreadsheets with all the relevant statistics are available.

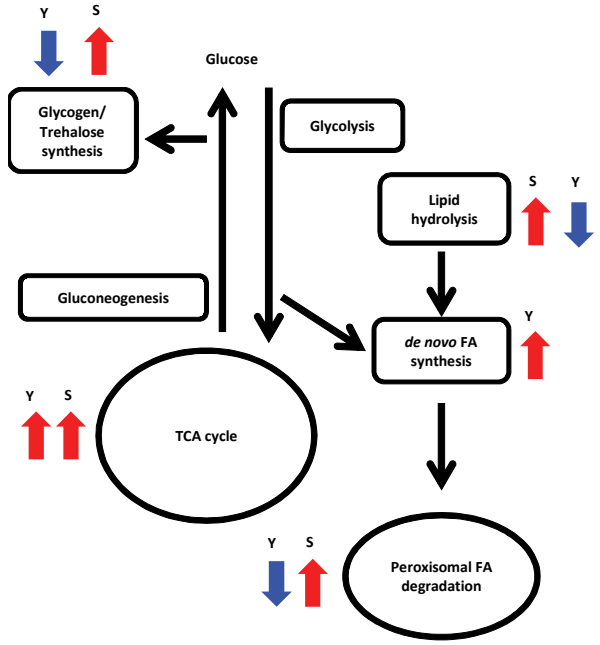

Figure 1: Differential expression of central metabolic pathways in log/stationary phase as deduced from transcriptome analysis. S = *S. cerevisiae,* Y = *Y. lipolytica*. Note that lipid (fatty acid) production is higher in *Y. lipolytica* during these growth phases.

Three types of analysis were performed: a) analysis of changes in gene expression in the various growth phases; b) comparative analysis of gene changes in *S. cerevisiae* vs *Y. lipolytica* in each of the growth phases (using previous microarray data for *S. cerevisiae* from the Sandmeyer group); and c) cluster analysis of *Y. lipolytica* gene expression patterns and MEME analysis of clustered promoter regions. Analysis (b) is the most relevant to this report, as motivated by the desire to understand how *Y. lipolytica* is able to produce more lipids than *S. cerevisiae*.

The metabolic pathways for central and fatty acid metabolism were constructed using CellDesigner (www.celldesigner.org) and fold change data were mapped to relevant genes with display by heat gradients. The software was used to cycle through and display differential expression in different growth phases; only select representative data is shown here. Comparison of *S. cerevisiae* (our actual biocatalyst) and *Y. lipolytica* (the organism that shows desirable behavior) between log and stationary showed that *S. cerevisiae* differentially up regulated genes related to glycogen and trehalose synthesis, gluconeogenesis, TCA cycle, and lipid hydrolysis, while *Y. lipolytica* down regulated genes related to glycogen and trehalose synthesis, and lipid degradation and up regulated TCA, and FA synthesis (Figure 1).

In addition, gene expression data were analyzed in the GeneSpring software (Silicon Genetics, Redwood City, CA). Statistical analyses using GeneSpring or genes imported following a CyberT

statistical analyses were also used to perform hierarchical and k-means clustering of the data. Seven general patterns of gene expression over the timecourse were identified for *Y. lipolytica* (data not shown). These were examined for over represented DNA sequence motifs and one such motif was identified. Representative promoters have been identified and are being transferred into expression vectors with the goal of controlling gene expression during different culture conditions.

(2) Analyze our biocatalyst during challenge with the target product

Reports in previous years have described our characterization of the growth sensitivity of *S. cerevisiae* BY4741 to short-chain (C_6, C_8 and C_{10}) carboxylic acids. Briefly, this sensitivity increases as chain length increases and is such that the presence of even 1mM of these compounds is sufficient to completely inhibit growth at pH 5.0. As we aspire to attain product titers higher than 1mM, this inhibition is concerning. Note that increasing the media pH decreases the magnitude of this inhibition. But because one of the benefits of working with yeast is its ability to thrive at a relatively low pH, simply increasing the media pH is not an acceptable solution to this problem. The goal of this omics analysis is to understand and address the problems posed by these carboxylic acids.

TF name	Function
Hap5	Activated during iron deprivation
Pho2	Activated during phosphate limitation
Met32	Activated during sulfur limitation
Cst6	Heat responsive
Haa1	Protective against weak organic acids such as acetate, but previously shown to have no effect on octanoic acid sensitivity
Hac1	Responds to ER stress
Ppr1	Regulates production of pyrimidines
Stb5	Responds to oxidative stress

Table 1: Transcription Factors (TF) with significantly altered regulatory activity during challenge with 0.3mM C8, as determined by Network Component Analysis (Yao Fu, Dickerson group)

We previously performed transcriptome analysis of *S. cerevisiae* BY4741 during mid-log growth in SDC media, pH 5.0, 30^0C with and without long-term exposure to 0.3mM C8. Note that this concentration of C8 is sufficient to decrease the specific growth rate by 25%. Identification of genes with significantly altered expression (data not shown) and regulators with significantly altered activity (Table 1) has led to several hypotheses regarding the mechanism of carboxylic acid toxicity.

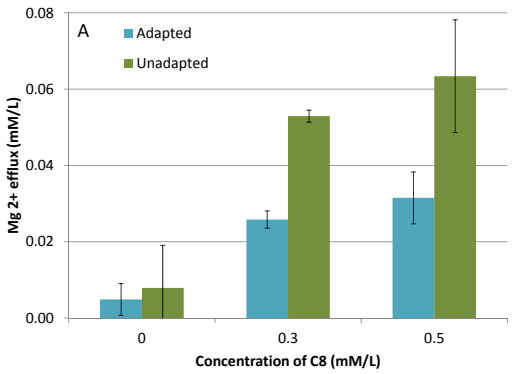

Figure 2: Leakage of Mg^{2+} in response to C8 challenge. Adapted cells were grown to log phase in the presence of C8 before C8 challenge.

The hypothesis that will be discussed here is that the carboxylic acids, due to their partial solubility in the fatty acid-based cell membrane, result in leakage of critical metabolites. This hypothesis is motivated by the fact that iron-, phosphate- and sulfur-starvation sensing regulators are activated in the +C8 condition (Table 1), but supplementation with additional iron, phosphate or sulfur does not increase C8 tolerance (data not shown).

Confirmation of this hypothesis was obtained by measuring the leakage of Mg^{2+} in the presence of C8. Mg^{2+} was used as a model metabolite due to the availability of commercial kits for its rapid measurement. We observed that not only did C8 challenge result in Mg^{2+} leakage in a dose-dependent manner (Figure 2), but cells that had adapted to C8 through prior exposure had decreased leakage. Note that there was no observed change in membrane fluidity for either whole cells or protoplasts in the presence of C8 (data not shown).

Therefore, the cells have a way of mitigating this leakage that is activated by C8 exposure. Identification and enhancement of this response could result in increased C8 tolerance. Since the membrane composition is likely to have a strong effect on membrane leakage, we measured the membrane fatty acid composition following growth with a variety of concentrations of C8 (data not shown). This analysis showed that the relative amount of saturated and unsaturated fatty acids did not change in response to C8, but there was an approximately 2-fold increase in C18:1 (oleic acid) and corresponding decrease in C16:1. Based on this result, we proposed that increasing the oleic acid content in the cell membrane may decrease C8-mediated Mg^{2+} leakage and growth inhibition.

Consistent with our proposition, increasing the oleic acid content in the membrane through media supplementation did increase tolerance to 1mM C8 (Figure 3), though there is no significant difference in Mg^{2+} leakage (data not shown). Current efforts are focusing on engineering the organism for increased oleic acid content in the membrane so that this compound will not be needed as a media supplement.

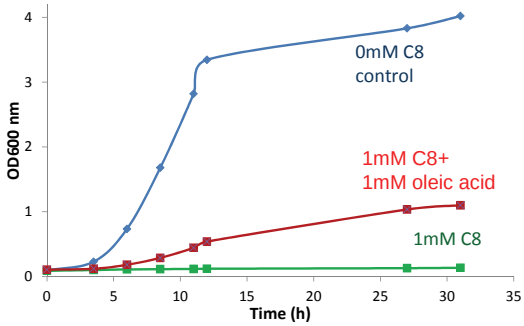

Figure 3: Supplementation of BY4741 with oleic acid increases tolerance to 1mM C8.

Other Relevant Work

Goal 1: Analysis of strains with increased fatty acid production.

Yazawa et al Yeast 2009 analyzed the transcriptome of *S. cerevisiae* engineered to produce polyunsaturated fatty acids (PUFA). Their transcriptome analysis led to many interesting findings, such as the fact that PUFA production is linked to the alkaline stress response. However, their analysis differed from ours in that they did not have a rigorous method for analyzing or visualizing their transcriptome data.

Goal 2: Analysis of inhibitory response

Previous researchers have looked at the growth response of yeast to fatty acid stress, but this stress was not investigated at a systems level. Instead, they screened insertion libraries or investigated specific enzymes, such as H^+-ATPase. This project differs in that we will be investigating the systems-level response to inhibition by and production of short-chain fatty acid and will be integrating this data with flux analysis.

Abbott et al FEMS Yeast Res (2007) used transcriptional analysis of the weak organic acid response to define a "generic" response during anaerobic growth. However, none of the genes in their defined generic response are perturbed in our datasets.

Plans for the Next Year

Simultaneous transcriptome and flux analysis. Upon identification of the appropriate condition for flux analysis, experiments will be conducted for simultaneous transcriptome and flux analysis. Analysis will focus on product toxicity while improvements are made in the carboxylic acid-producing strain. Long-term plans include simultaneous transcriptome and flux analysis of the producing strain, both for the carboxylic acid test bed and the pyrone test bed.

Toxicity transcriptome follow-up: The work described above shows that supplementation with oleic acid (C18:1) increases tolerance to C8 and increases C18:1 content in the media. Metabolic engineering strategies will be used to increase C18:1 content in the membrane; these methods have been previously reported in the literature to enable C18:1 content as nearly 70% of the membrane.

Expected Milestones and Deliverables

Simultaneous transcriptome/flux analysis during moderate carboxylic acid challenge.

Transcriptome and flux analysis of the engineered carboxylic acid-producing and pyrone-producing strain(s) – this is a long-term goal.

Member Company Benefits

It is anticipated that this research project will generate valuable know-how and/or intellectual property for member companies. This includes a general framework for using omics analysis to identify metabolic bottlenecks and toxicity of substrate or product compounds, something that is very relevant to cellulosic ethanol and next-gen biofuels.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.4A -** Flux Analysis in *E. coli*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Jacqueline V. Shanks	**02/17/2012**	**03/01/2011 to 02/29/2012**

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Statement of Project Goals

The goal of the project is to construct metabolic flux maps for *E. coli*, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

Metabolic flux maps are an integral part of the metabolic engineering design cycle to construct strains that produce carboxylic acids and pyrones, the intermediate biochemicals of two CBIRC test beds that require catalysis via Thrust 3 to make the α-olefins and dienes or dienoic acids, respectively. Metabolic fluxes are an important physiological characteristic, providing a global perspective of the integrated functioning between levels of transcripts, proteins, and metabolites to cellular phenotype. Metabolic flux analysis identifies potential bottlenecks in the reaction network that limit production of the target compound. These bottlenecks are then genetically engineered out in the next metabolic engineering cycle.

Fundamental Barriers and Methodologies

A key barrier in the overall goals of Thrust 2 will be to shorten the metabolic engineering cycle. Since flux plays an integral role in the metabolic engineering cycle, this means a quick turnaround time for flux analysis results to the strain construction and bioinformatics projects. Fundamental barriers for metabolic flux analysis lie in (1) the validation of the flux map, (2) in deciding the right metabolic flux analysis mapping tool for the application, and in (3) the correct basis of the metabolic flux results to integrate into the bioinformatics framework for comparison to other data sets. For validation of the flux map, the network topology and nomenclature is coordinated with the CBIRC-enhanced Ecocyc

pathway database as well as using large scale isotopomer models, and CBiRC generated 'omics data. In deciding the tradeoff in the time intensive but information rich comprehensive flux analysis versus a more high-throughput "fluxomics" method (which either only uses partial labeling information to obtain a flux map or correlates labeling information via a bioinformatics approach) an assessment of conventional MFA and fluxomics MFA, was benchmarked with comprehensive MFA, so that a design strategy can be assessed so that more strains can be characterized at the level needed.

Achievements

Octanoic Acid Toxicity MFA of MG1655

Comprehensive and reproducible flux maps of *E. coli* MG1655 under control conditions and toxicity challenge (35mM octanoic acid) in M9 media were obtained. Aerobic batch cultures were conducted in a Multifor system with working volume 400 ml, at 37°C, with 650rpm agitation and pH control to 7.0. The dissolved oxygen (DO) level was kept above 50% saturation during the entire fermentation to ensure aerobic conditions. The 35mM C8 exposure caused ~53% inhibition on the growth rate. The acetate yield and specific acetate production rate increased by 1.82 fold and 1.34 fold, respectively, in comparison with those in control, while the growth rate, biomass yield, and specific glucose uptake rate decreased by 2.02 fold, 1.53 fold, and 1.35 fold respectively. A one-tail T-test of the 3 biological replicates shows the differences in the phenotype are statistically significant (p value < 0.05).

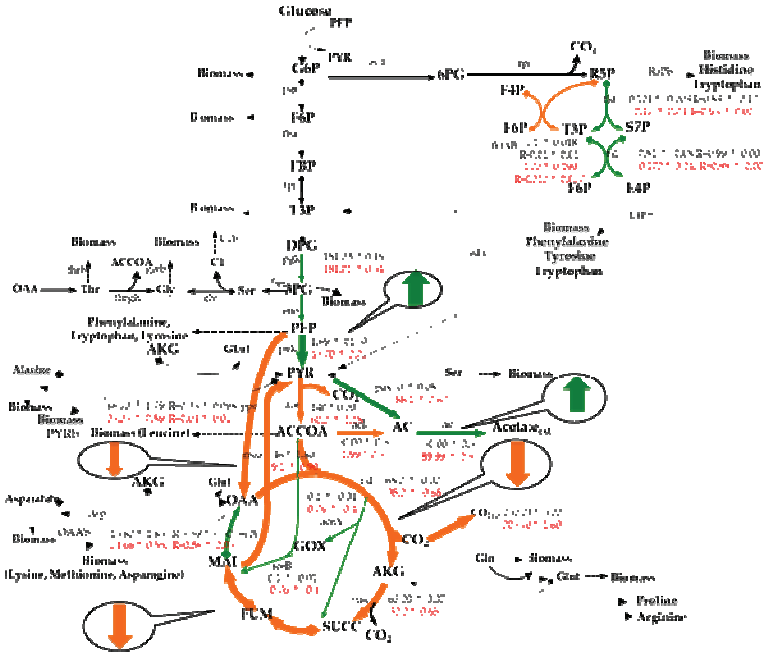

Figure **1.** Flux map for both control condition and 35mM C8 stress condition obtained from NMR2Flux using NMR data. The values in black are control condition, the values in red are C8 stress. The R values indicate reversibility for each of the reversible reactions. Flux values are normalized to 100 moles of glucose consumed.

The MFA under C8 exposure revealed the TCA cycle flux is inhibited by ~ 44%, malic enzyme pathway is inhibited by ~ 80%, phosphoenolpyruvate carboxylase pathway ('ppc') is inhibited by ~ 60%, CO_2 production rate decreases by 18% and pyruvate dehydrogenase pathway is inhibited by ~50%. Meanwhile, the pyruvate oxidase (pox) flux becomes active under C8 stress, the malate dehydrogenase pathway in TCA cycle increases by ~55%, the extracellular acetate flux increases by ~ 80%. Furthermore, the oxygen uptake rate decreases under C8 stress. These flux changes, in conjunction with our transcriptomic and proteomic data, suggest that carboxylic acids disrupt the membrane and electron transport chain. Testing of these hypotheses is discussed in project T2-3A.

^{13}C Metabolic Flux Analysis of ML103 pXZCO04 in Shaker Flasks

^{13}C labeling experiment was conducted in shaker flasks. M9 medium with 1.5% glucose was used for the experiments with initial pH adjusted to 7.0. The sole carbon source in this experiment was a mixture of ^{13}C labeled glucose (20% uniform labeled glucose and 80% 1-position-labeled glucose). Physiological differences of ML103 pXZCO04 under control and fatty acid producing condition (with IPTG induction) are shown in Table 1. No significant difference in growth rate was observed between the control condition and fatty acid producing condition. Under 1mM IPTG induction, glucose uptake rate and acetate yield decreased by 20% and 13% respectively. Fatty acid yield was 4-fold higher under IPTG induction.

Table 1. Physiology properties of ML103 pXZCO04 under control and IPTG induced condition

	0mM IPTG	**1mM IPTG**
Growth rate (hr^{-1})	0.381 ± 0.001	0.384 ± 0.001
Glucose uptake rate (mole/g/hr)	7.22 ± 1.18	5.80 ± 0.84
$Y_{acetate/glucose}$ (mole/mole)	0.540 ± 0.034	0.470 ± 0.054
$Y_{fatty\ acid/glucose}$ (mole/mole)	0.011 ± 0.002	0.041 ± 0.010
% of max theoretical yield	4.5 ± 0.9 %	16.3 ± 3.8 %

For a quick flux map assessment, simulations using GC-MS isotopomer and extracellular measurements produced the flux maps of ML103 pXZCO04 strain under induced and uninduced conditions (Figure 2). The maps showed increases in the glycolysis pathway, and decreases in the ED pathway and TCA cycle activities. TCA cycle decreased by 30% mainly because carbon was channeled to acetyl-coA for fatty acid production. Comprehensive flux maps from NMR-based fermentor experiments confirmed the flux reduction in the TCA cycle under fatty acid production.

From inspection of these results, we hypothesized disruption of the TCA cycle may further enhance carbon flow towards fatty acid biosynthesis pathway. However, we also observed flux reduction in TCA cycle under octanoic acid toxicity. Therefore, we conducted toxicity studies of hexadecanoic acid on ML103pXZCO04. The growth rate decreased by 20% at 0.5 mM of C16 fatty acid, indicating toxicity. But, the growth rate in ^{13}C experiments showed no difference between control and fatty acid overproducing condition. Thus, flux reduction in TCA cycle may be related to fatty acid production rather than toxicity.

We then chose a current CBiRC strains that would be reduced in TCA cycle function. We extracted and transformed pXZCO04 plasmid to MLK163, which has an additional *sucC* gene knockout. The *sucC* gene is responsible to produce succinate from succinyl-CoA. The titer under rich LB media with 1.5% glucose achieved 3.6g/L, a ~2-fold increase in 48 hours compared to ML103 pXZCO04 (Figure 3). Determining the fatty acid titer under M9 media with 1.5% glucose is underway.

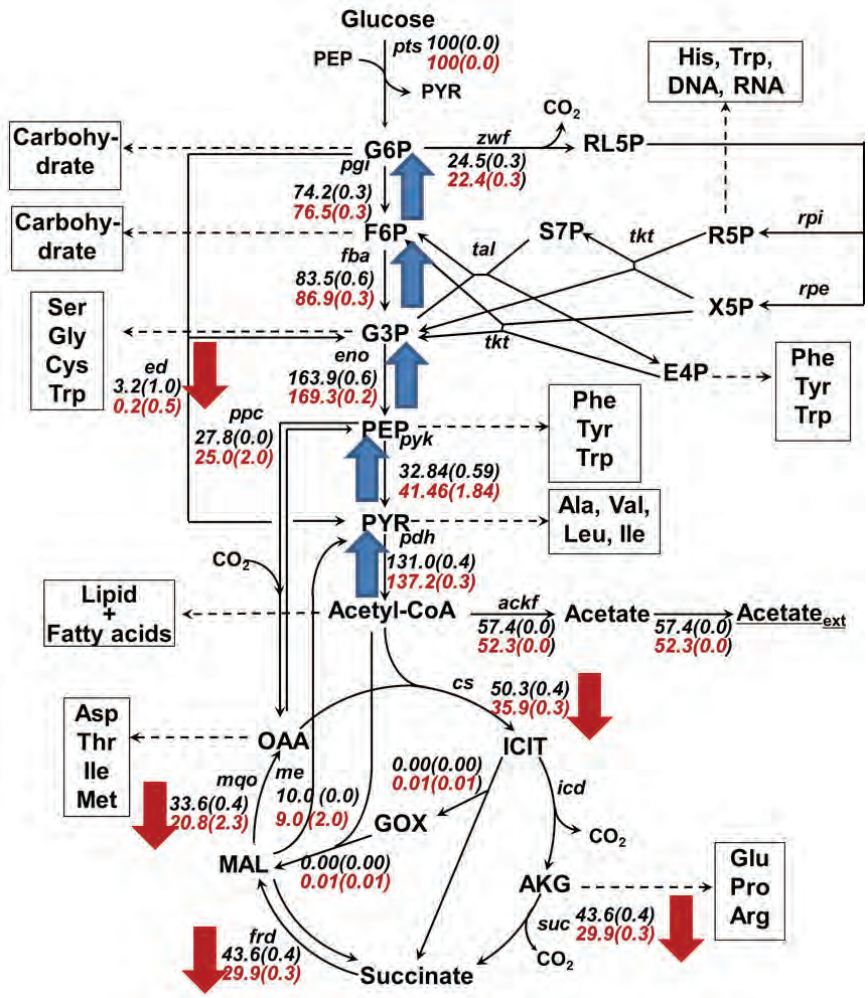

Figure **2.** Flux map of ML103pXZCO04 for both control condition and fatty acid overproducing condition, the values in black are control condition, the values in red are fatty acid producing. All the flux values are normalized to 100 mols of glucose consumed.

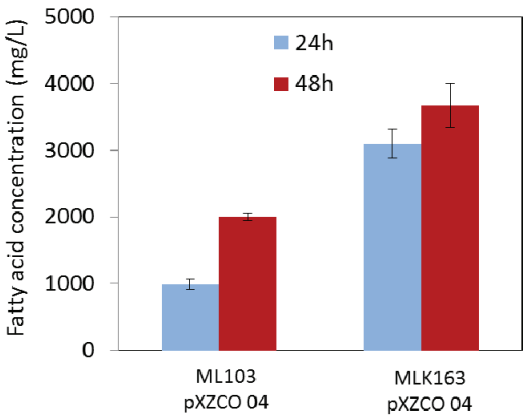

Figure 3. Fatty acid concentration of ML103 pXZCO04 (Δ*fadD, thioesterase*++) and MLK163 pXZCO04(Δ*fadD*, Δ*sucC, thioesterase*++) strains in LB media with 1.5% glucose.

Integrating OPTforce and Flux Data for Genetic Manipulation Prediction

Metabolic flux map for wild type MG1655 under aerobic condition has been performed and the flux results were given incorporated into OPTforce simulations using genome-scale *E. coli* model *i*AF1260 as a basis. With flux constraints, OPTforce suggested a cascade of genetic interventions to attain high fatty acid with specific carbon chain lengths, from C6 – C16. Based on the OPTforce predictions, ML103pXZ18z strain, in which *fabZ* gene in fatty acid biosynthesis cycle is overexpressed, was chosen for the next iteration in strain improvement for C14-C16 fatty acids. We observed that this strain was able to produce ~2.3g/L fatty acids in defined M9 media with 1.5% glucose after 72 hours (Figure 4) which is about five times as much as that of ML103pXZCO04 (~0.4g/L) . We are currently developing flux maps for ML103pXZ18z under fatty acid overproducing condition. The flux result will serve as the input for OPTforce for its second round simulation for prediction of further genetic manipulations. By completing this, we will show an integration of analytical, computational and metabolic engineering to close up engineering loop for overproducing C14-C16 fatty acids in *E.coli***.** The principles learned here will then be transferred to shorter chain fatty acids.

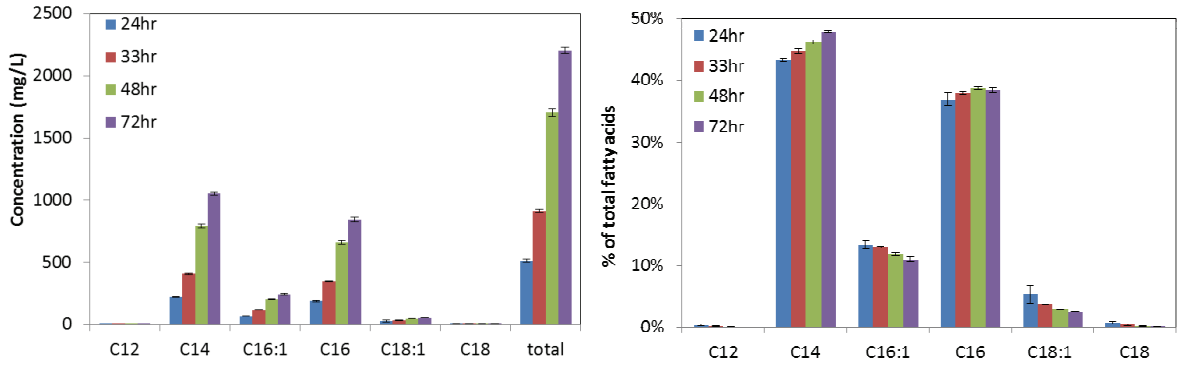

Figure 4. Carboxylic acid production and its composition by ML103pXZ18z strain in a defined M9 medium with 1.5% glucose. Fatty acid titer reached 2.5g/L after 72 hours and most fatty acids were 14- or 16-carbon fatty acids.

Other Relevant Work

Flux analysis methods in yeast and plant systems leverage flux tool development for *E. coli.*

Plans for the Next Year

CBiRC generated flux data (ISU) is used in Optforce (Penn State) and FBA models made from BioMart (ISU) to predict necessary flux alterations for optimal productivity of a given chain length. MFA of *E. coli* strains engineered at Rice University to improve fatty acid synthesis will be compared with wild-type and in silico predictions. MFA will be performed for strains engineered for new testbed products. Finally, we are collaborating with the Bioinformatics project for incorporation of flux data into BioMart for ease of comparison of transcript and other physiological data.

Expected Milestones and Deliverables

Predictions of flux design targets for fatty acid production as the chain length changes from C16 to C6. Integrated metabolic flux platform (simulation and experiment) and biosystems design of *E. coli* strains engineered to improve short chain fatty acid synthesis.

Member Company Benefits

CBiRC member companies, especially the biotechnology start-ups, have expertise in construction of strains but do not have the resources for such detailed flux analysis. The expertise required for experimental and computational flux analysis is not trivial and in short supply. Our team has extensive experience in

experimental metabolic flux analysis, constructing genome-scale metabolic models and computational metabolic flux analysis and optimization procedures. The integrated flux platform is being demonstrated in CBiRC testbeds, and will create a powerful tool for strain design and optimization.

Commercialization / Technology Transfer

We are actively engaging with Center member companies, including start-ups, in discussion of the integrated flux platform with their research needs.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.4B -** Flux Analysis in *S. cerevisiae*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Jacqueline V. Shanks	**02/17/2012**	**03/01/2011 to 02/29/2012**

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Ali Zomorrodi, Department of Chemical Engineering, The Pennsylvania State University
Assistant Scientists:
Jong Moon Yoon, Department of Chemical and Biological Engineering, ISU

Statement of Project Goals

The goal of the project is to construct metabolic flux maps for *S. cerevisiae*, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

Project's Role in Center's Strategic Plan

Metabolic flux maps are an integral part of the metabolic engineering design cycle to construct strains that produce carboxylic acids and pyrones, precursor chemicals that require catalysis via Thrust 3 for the synthesis of α-olefins, dienes, and other compounds. Metabolic fluxes are an important physiological characteristic, providing a global perspective of the integrated functioning between levels of transcripts, proteins, and metabolites to cellular phenotype. Metabolic flux analysis identifies potential bottlenecks in the reaction network that limit production of the target compound. These bottlenecks are then genetically engineered out in the next metabolic engineering cycle.

Fundamental Barriers and Methodologies

A key barrier in the overall goals of Thrust 2 will be to shorten the metabolic engineering cycle. Since flux plays an integral role in the metabolic engineering cycle, this means a quick turnaround time for flux analysis results to the strain construction and bioinformatics projects. Fundamental barriers for metabolic flux analysis lie in (1) the validation of the flux map, (2) in deciding the right metabolic flux analysis mapping tool for the application, and in (3) the correct basis of the metabolic flux results to integrate into the bioinformatics framework for comparison to other data sets. For validation of the flux map, the network topology and nomenclature is coordinated with the Bioinformatics project that mines *S. cerevisiae* data. In deciding the tradeoff in the time intensive but information rich comprehensive flux analysis versus a more high-throughput "fluxomics" method (which either only uses partial

labeling information to obtain a flux map or correlates labeling information via a bioinformatics approach) an assessment of conventional MFA and fluxomics MFA, was benchmarked with comprehensive MFA, so that a design strategy can be assessed so that more strains can be characterized at the level needed.

Achievements

^{13}C Labeling Experiment for Octanoic Acid (C8) Stress and Control Conditions

In the previous report, we presented flux maps of *S. cerevisiae* using 0.25mM octanoic acid in SD minimal media in shake flasks. In the current year, we conducted larger-scale and controlled reactor experiments. Aerobic batch cultures were conducted in a Multifor system with working volume 400 ml, at 30°C, with 600 rpm agitation and pH control to 5.0. The dissolved oxygen (DO) level was kept above 50% saturation during the entire fermentation to ensure aerobic conditions. For a quantitative flux map, a defined medium is necessary. Thus, the amino acid concentrations for carbon-limited growth were determined. A concentration of 10g/L glucose meets carbon-limited growth with 5X amino acid supplementation in SD minimal medium, where 1X amino acid represents 20mg/L histidine, 20mg/L methionine, 20mg/L uracil and 60mg/L leucine. For labeling experiments, the glucose composition was 20% uniformly labeled and 80%1-^{13}C labeled glucose. Extracellular metabolite secretion rates and glucose uptake rate were determined using HPLC. Isotopomer fractions of amino acid were obtained from NMR.

The yield coefficients for biomass formation and fermentation product secretion on glucose consumption are presented in Table 1 during aerobic batch fermentation of *S. cerevisiae*. The cells exhibited respiratory-fermentative metabolism with secretion of ethanol, acetate and glycerol. Under C8 fatty acid inhibition, cells grew slower with lower specific growth rate (reduced by 25%) and a higher specific glucose uptake rate. Ethanol and acetate yield increased by 10% and 2-fold respectively, coupled with lower biomass yield of 41% under C8 fatty acid inhibition. However, glycerol yield decreased 7-fold with C8 fatty acid inhibition.

The isotopomer measurement from NMR revealed no significant difference for most of the amino acid fragment under C8 stress. We observed difference in isotopomer fractions in proline-β, glutamate-β , lysine-δ and lysine-γ (Figure 2). These amino acids are precursors for α-ketoglutarate in the TCA cycle, which will contribute to TCA cycle activities perturbation.

MFA of BY4741 for both octanoic acid (C8) stress and control conditions

With extracellular metabolite rates, amino acid isotopomer measurements via NMR, and biomass composition data, flux maps of *S. cerevisiae* were developed with NMR2Flux. Figure 3 showed pentose phosphate (PP) pathway flux of ~20 for both ethanol control and C8 fatty acid case. The relative PP fluxes and low activity through the TCA cycle have been observed for other *S. cerevisiae* wild-type strains under similar physiological conditions [Gombert *et al* (2001)].

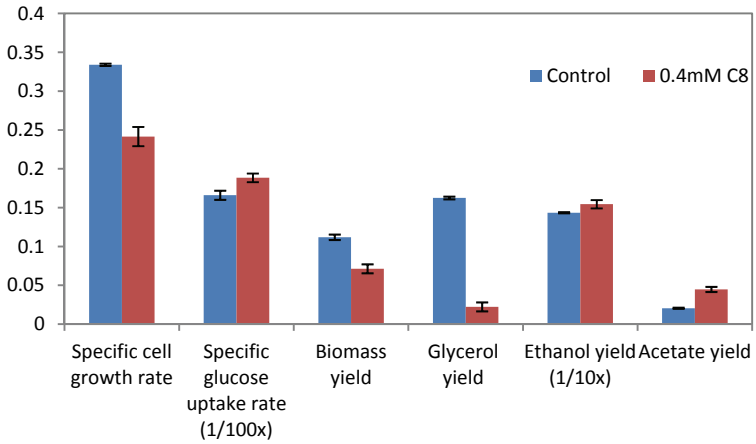

Figure 1. Phenotype analysis of *S.cerevisiae* under 0.4mM C8 fatty acid inhibition. Specific glucose uptake rate and ethanol yield were scaled down 100x and 10x respectively. Unit: specific cell growth rate, hr-1; specific glucose uptake rate, mmol/g DCW.hr; biomass yield, g DCW/g glucose; fermentation product yields, mol/mol glucose

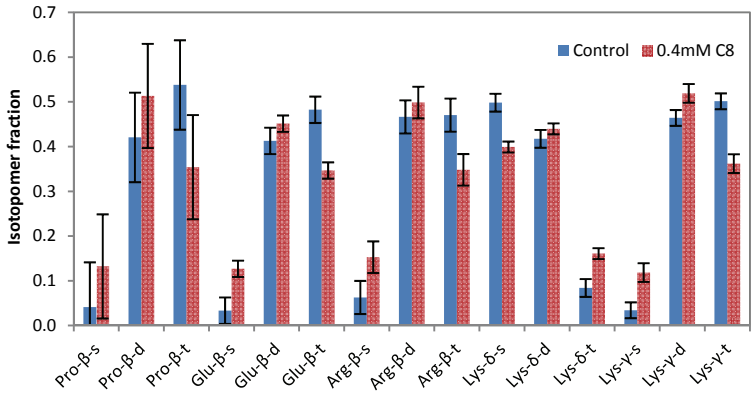

Figure 2. Isotopomer difference of several amino acids was detected in NMR measurements, Noticibly, proline, glutamate, arginine and lysine are all originated from α-ketoglutarate in mitochondrion which might contribute to the flux difference in TCA cycle.

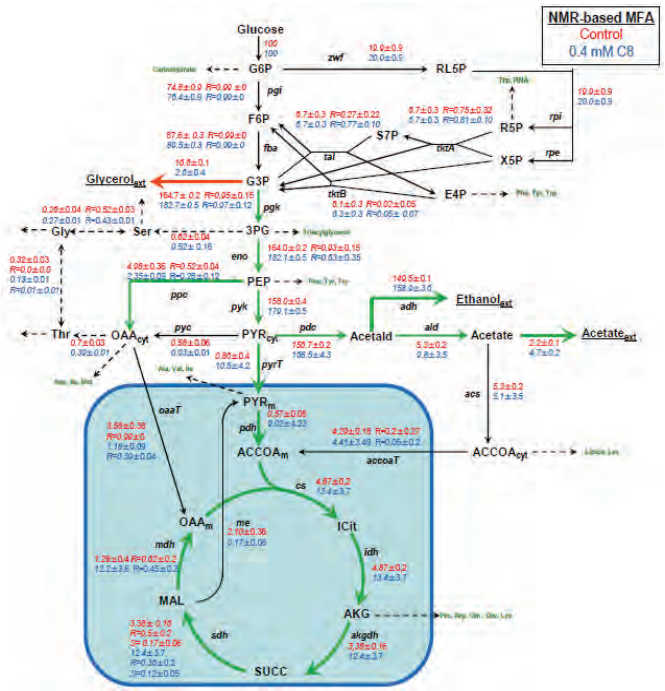

Figure 3. Metabolic flux map of *S. cerevisiae* BY4741 under aerobic conditions with ethanol control and 0.4mM C8 fatty acid inhibition obtained from simulation with NMR data. The blue shaded box represents mitochondrion compartment, letter R represents reversibility.

Compared with control, the fluxes through glycolysis pathway were mostly higher under fatty acid stress due to lower biomass efflux (carbohydrates and amino acids) and glycerol efflux. Glycerol plays important roles in physiological processes such as combating osmotic stress, managing cytosolic phosphate levels and maintaining the NAD+/NADH redox balance. Since extracellular octanoic acid might disrupt the function of glycerol production, knock-out strains of key genes in glycerol formation were challenged with 0.3 mM of C8 in the SD minimal media. However, the knock-out strains, *S. cerevisiae* (GDP1-KO) and *S. cerevisiae* (GDP2-KO), exhibited the same reduction in growth as observed in the wild-type strain (*data not shown*). Additional knock-out strains (GPP1-KO and GPP2 KO are currently being tested.

Furthermore, under C8 stress, TCA cycle activities were 3-fold higher and pyruvate transport flux into mitochondrion was 10-fold higher, perhaps reflecting an increased need for ATP. These flux results are in contrast to those observed with *E. coli* exposed to octanoic acid stress. More discussion of the hypotheses tested for yeast toxicity is included in T2.3B - Omics Experiments in *S. cerevisiae*. The transcriptomic and fluxomic groups are currently trying to reconcile the observations in the data.

Integrating OPTforce and Flux Data for Pyrone Production

Metabolic flux maps for wild type BY4741 under aerobic conditions has been performed and the flux results are being incorporated into OPTforce simulations using an improved *S. cerevisiae* genome-scale model iMM904 as a basis. With flux constraints, OPTforce will suggest a cascade of genetic interventions to attain a higher yield of pyrone. These predictions will guide metabolic engineering design for yeast for pyrone production (see Projects T2.1B and T2.2B).

Other Relevant Work

Flux analysis methods in plant and *E. coli* systems leverage flux tool development for microbial systems.

Plans for the Next Year

MFA of *S. cerevisiae* strains engineered at University of Irvine to improve short-chain fatty acid synthesis will be compared with wild-type. Flux analyses of strains exposed to toxic levels of short-chain fatty acids are being performed in parallel with transcriptomic experiments and bioinformatic analysis in order to enhance tolerance to C6 and C8 fatty acids. CBiRC generated flux data (ISU) is used in Optforce (Penn State) to predict necessary flux alterations for optimal productivity of pyrones in engineered *S. cerevisiae* strains (Irvine). MFA will be performed for strains engineered for new testbed products.

Expected Milestones and Deliverables

With collaboration with the omics project, we will perform transcriptomic experiments in conjunction with the ^{13}C labeling experiment to help test hypotheses of mechanisms of fatty acid toxicity.

Integrated metabolic flux platform (simulation and experiment) and biosystems design of *S. cerevisiae* strains engineered to improve short chain fatty acid or pyrone synthesis.

Member Company Benefits

CBiRC member companies, especially the biotechnology start-ups, have expertise in construction of strains but do not have the resources for such detailed flux analysis. The expertise required for experimental and computational flux analysis is not trivial and in short supply. Our team has extensive experience in experimental metabolic flux analysis, constructing genome-scale metabolic models and computational metabolic flux analysis and optimization procedures. The integrated flux platform is being demonstrated in CBiRC testbeds, and will create a powerful tool for strain design and optimization.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.5A** – Bioinformatics in *E. Coli*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
J. A. Dickerson	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Faculty: Julie A. Dickerson (ISU), Laura Jarboe (ISU), Ramon Gonzalez (Rice University), Jackie Shanks (ISU), Ka-Yiu San (Rice University)

Graduate Students: Erin Boggess (ISU), Al Yao Fu (ISU), Liam Royce (ISU), Jesse Walsh (ISU)

Undergraduate Students: Kara Moeller (ISU)

Statement of Project Goals

Develop models to integrate in-house omics data with existing databases to provide a system-wide view of the production strains. Develop tools based on a systems-wide approach to provide insights and suggestions for further strain improvement.

Project's Role in Center's Strategic Plan

The bioinformatics tools developed in this project will provide a new model for improving strains and achieving optimized product production. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins and dienes by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

Meaningful data integration across heterogeneous data sources is difficult to achieve as the importance and reliability of data sources is unknown at this time. Additional problems include incomplete and uncertain data on the structure of the metabolic networks under different conditions.

Achievements

In order to promote greater sharing of knowledge and improve interdisciplinary training for the graduate students, the *E. coli* omics experiments and bioinformatics teams have been meeting weekly since the beginning of the project. These discussions have reviewed key pathways in E. coli central metabolism, bioinformatics tools for omics data, and of metabolic flux analysis. The bioinformatics team has been using existing *E. coli* datasets to explore different bioinformatics tools for analysis of gene regulatory networks.

The bioinformatics team created a novel method for discovering new links of the transcription factor network that combines regulatory network learning methods with Network Component Analysis. This information is being used to assess the effects of interventions suggested by the pathway optimization team.

Integrated Pathway Database for *E. coli*

The purpose of this task is to provide a flexible and integrated omics data resource for both storage and retrieval of *E. coli* metabolic data necessary for Thrust 2 research. The database can be accessed via the web. A systems approach combines the various omics data into a single entity is necessary in order to answer the complex questions that arise when trying to manipulate the metabolism of *E. coli*. The proposed solution is to create a flexible, integrated relational database. This database is pathway centric with additional annotations from various other omics datasets, while also allowing for a custom tagging system which will manage data produced by Thrust 2 biologists. We have updated the underlying pathway tools to the latest version and have integrated in the regulatory links predicted by our algorithms. In addition, we have developed a set of tools which allow direct manipulation of frame objects in the PGDB through the Pathway Tools API. These tools allow the user to create or edit changes on a large scale and track the changes made. We have also developed a set of custom web services which can be used as “canned” queries against the local EcoCyc database. Queries include gene ID converter, with the ability to look up alternate IDs for gene, given a common name, ID, Blattner number, or microarray probe name.

Automatic Model Generation for Flux Analysis

The Dickerson lab has developed tools to generate models based on either whole organism or individually selected pathways and reactions from PGDBs. New features include automatically working with boundary, diffusion, and generalized reactions. Diffusion is based on a similar strategy used in making the iAF1260 model, where molecules smaller than 600 daltons found in the periplasm are assumed to permeate the outer membrane freely. In process of verifying models to help determine the accuracy of the reconstruction. EcoCyc uses generalized reactions such as "carbohydrate (extracellular) --> carbohydrate (periplasm)". These general reactions must be instantiated in order for metabolic networks to connect properly. For example, lactose has its own transport reaction from cytoplasm to periplasm, but falls under the carbohydrate category when transported between periplasm and extracellular. We are developing automated methods for working with the reactions and bringing them into the model.

Exploring Gene Regulation in *E. coli.*

Short chain fatty acid toxicity

Previous work:

Based on the microarray experiments of C8 fatty acid stress from experiments last year, we predicted TFAs using NCA. Three different initial TF-gene regulatory network topologies, including GRNs obtained from RegulonDB 7.0, RegulonDB 7.0 + 125 GTRNetwork Predicted Links, and RegulonDB 7.0 + 381 GTRNetwork Predicted Links, are used as inputs to predict three sets of TFAs. The changing directions of TFAs are corrected using the D-NCA algorithm. Significantly changed TFAs are identified using a t-test. Further experiments proved that the intracellular pH of *E. coli* under C8 fatty acid stress experiment is decreased. Another set of significantly changed TFAs under low pH conditions is predicted from microarray data from the M3D database. We compared the significantly changed TFAs under C8 fatty acid stress and the significantly changed TFAs under low pH. A set of TFs changed specifically under C8 fatty acid stress but not in response to low PH. We hypothesized that those TFs mainly respond to C8 fatty acid stress. These TFs are shown in our publication: Fu, Y., L.R. Jarboe, J.A. Dickerson, "Reconstructing genome-wide regulatory network of *E. coli* using transcriptome data and predicted transcription factor activities," *BMC Bioinformatics*, **12**:233, 2011, doi:10.1186/1471-2105-12-233.

Current Efforts

293 genes are regulated by the significantly changed TFs described above. These 293 genes affect 74 pathways and include 81 membrane related genes. Recent experiments performed by the Jarboe Lab showed that the membrane damage might be the crucial cause of fatty acid toxicity; thus, those 81 membrane related genes that are controlled by the TFs with significantly changed activities might be important.

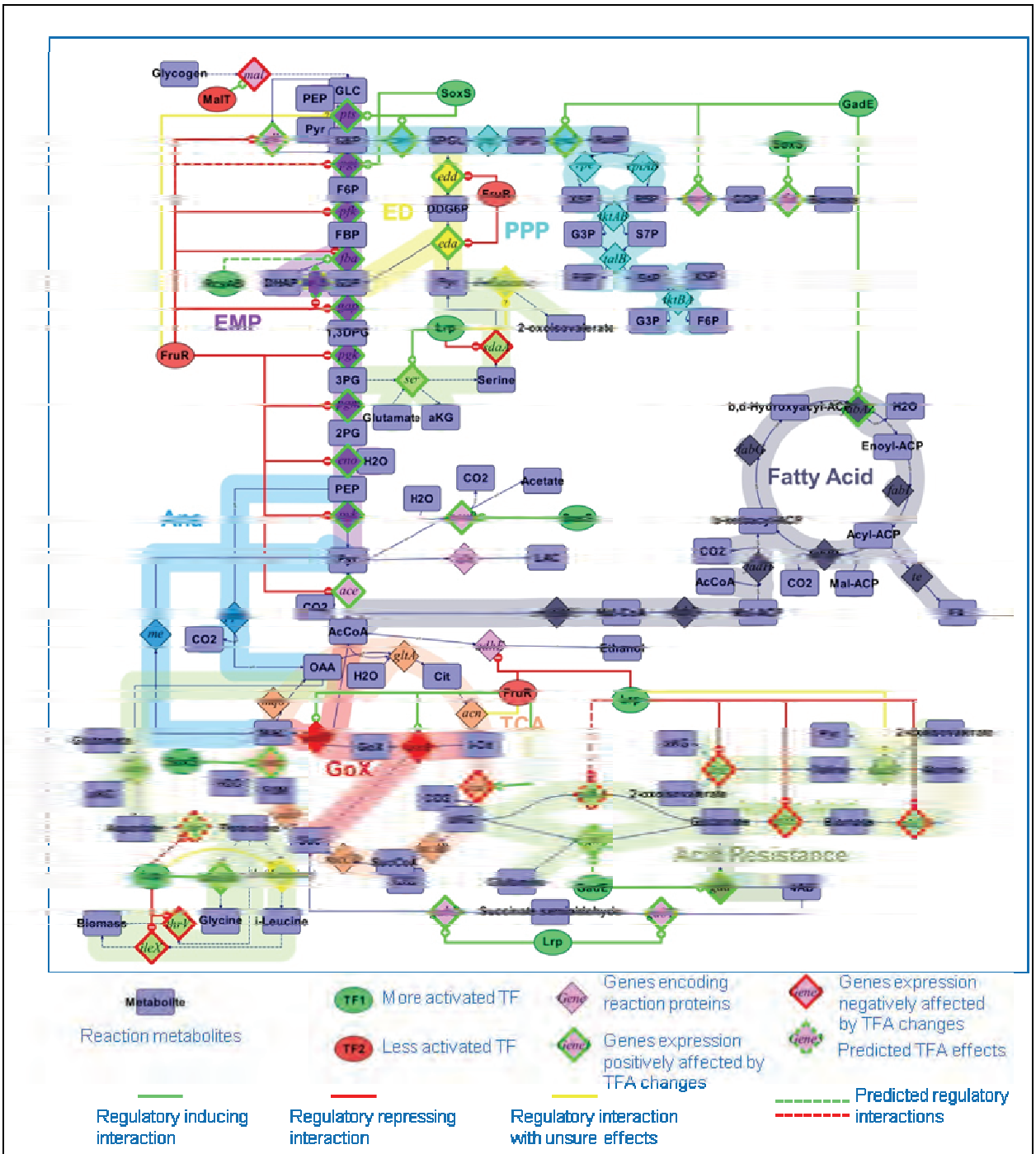

Figure 1 Regulatory effects of these activities significantly changed TFs to the central carbon metabolism

These regulatory effects match the flux analysis results which show that when the Glycolysis, ED, and pyruvate to acetate pathways are turned up, the TCA cycle is turned down. These flux outcomes are in agreement with the regulatory effects of the active TFs. Specifically, Glycolysis and ED pathway genes are turned on by reduced activity of repressor FruR to promote the utilization of glucose, and one of the TCA cycle genes icd is negatively affected by FruR. The reaction to generate acetate from pyruvate is turned on by the oxidative stress response regulator SoxS, and pulls some carbon flow from glycolysis.

Metabolic Engineering

Task 1a has produced engineered strains showing high yields of free fatty acids. The most promising mutations include overexpressing *fabZ,* and knocking out *sucC*, and the second best strain also includes a knockout of *fabR.* Overexpression of *fadR* also gave promising results. We simulated these mutations to gain a better understanding of the regulatory effects of these mutations. Figure 2 shows that FabR has only two target genes in the super pathways of fatty acid biosynthesis, and may be a good target for engineering to increase the production of fatty acid. FadR can negatively affect the TCA cycle to reserve more carbon flow for fatty acid synthesis. It also represses the fatty acid beta-oxidation pathway. The GTRNetwork prediction shows that FadR also downregulates the arginine degradation pathway. Figure 3 show the regulators of *fabZ* and *sucC. SucC* is regulated by many global regulators that regulate over 100 genes. *FabZ* is regulated by GadE and CpxR, where GadE is shown being more activated under C8 fatty acid stress.

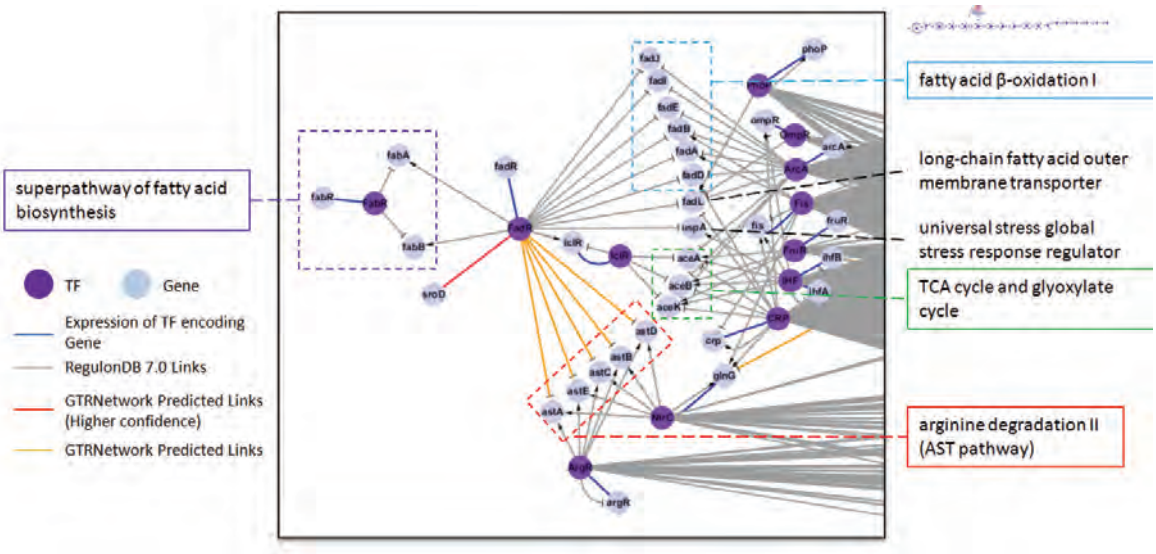

Figure 2 Regulatory network of FabR and FadR

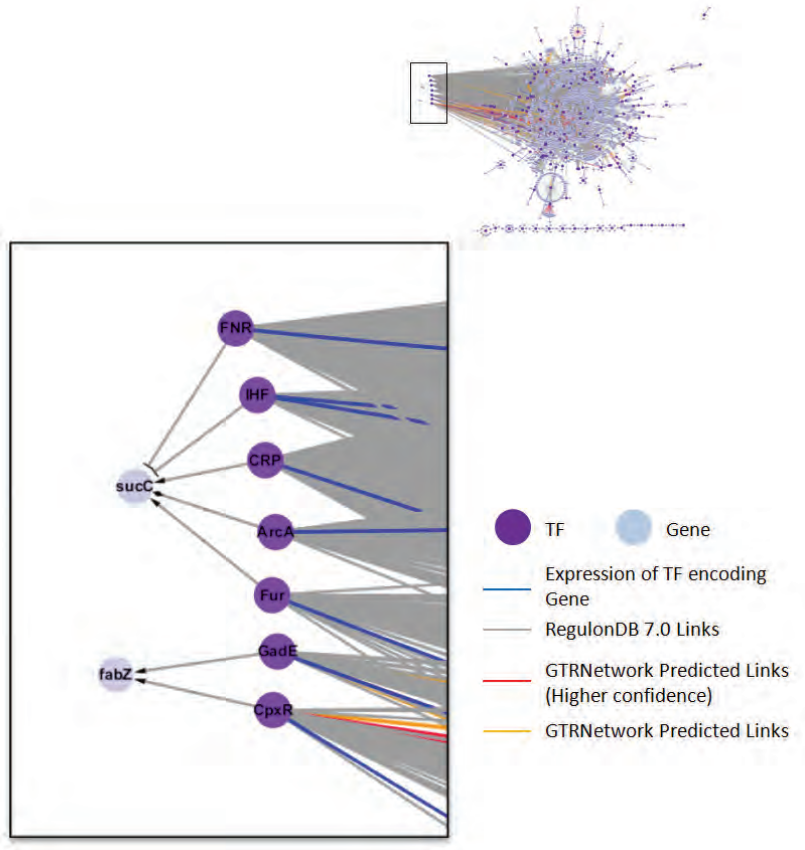

Figure 3 Regulators of *sucC* and *fabZ*

Other Relevant Work

Relevant similar work is also being conducted within CBiRC using yeast as the model microbial system. Many studies have been done to learn gene regulatory networks from microarray data and we are comparing our results for this data. Our work will integrate networks from different sources and combine them with pathway data information to get a more complete picture of interactions.

Plans for the Next Year

The Dickerson lab will continue to work with our collaborating labs to develop new tools for omics data analysis focused around the structure of the metabolic network of *E. coli*. These tools will be paired with analytical models for the systems of interest and visualized using MetNetGE.

Expected Milestones and Deliverables

- Integrate flux data and transcriptome data into a more complete *E. coli* metabolic model.
- Improve methods for the detection of indels in RNAseq datasets for determining the effects of directed evolution.

Member Company Benefits

The visualization tools and the methods for analyzing metabolic networks would be useful for scientists at these companies to quickly assess the results of large-scale omics investigations.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.5B –** Bioinformatics in *S. cerevisiae*

Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Eve Wurtele	**2/17/2012**	**03/01/2011 to 02/29/2012**

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Suzanne Sandmeyer, Department of Biological Chemistry, University of California Irvine
Research Scientists: Ling Li Department of Genetics, Cell and Development Biology, Iowa State University,
Postdoctoral Scholars: Tarek Najdi Department of Biological Chemistry, University of California Irvine; Wang Yi Department of Genetics, Cell and Development Biology, Iowa State University
Graduate Students:
Jon Hurst, Department of Genetics, Cell and Development Biology, Bioinformatics and Computational Biology Program, Iowa State University, Yves Sucaet, Department of Genetics, Cell and Development Biology, Bioinformatics and Computational Biology Program, Iowa State University, Alexis Campbell, Dept. of Biochemistry, Biophysics and Molecular Biology

Statement of Project Goals

Identify new genes that regulate polyketide metabolism in yeast. Develop models to integrated in-house 'omics data with existing databases to provide a system- wide view of the production strains. Develop tools based on a systems-wide approach to provide insights and suggestions for further strain improvement, and to systematically optimize yeast performance for diversion of carbon to synthesis of carboxylic acids and pyrones.

Project's Role in Center's Strategic Plan

The bioinformatics tools developed in this project, and the genes identified, and models developed, provides a new approach for improving strains and achieving optimized product production of polyketides.

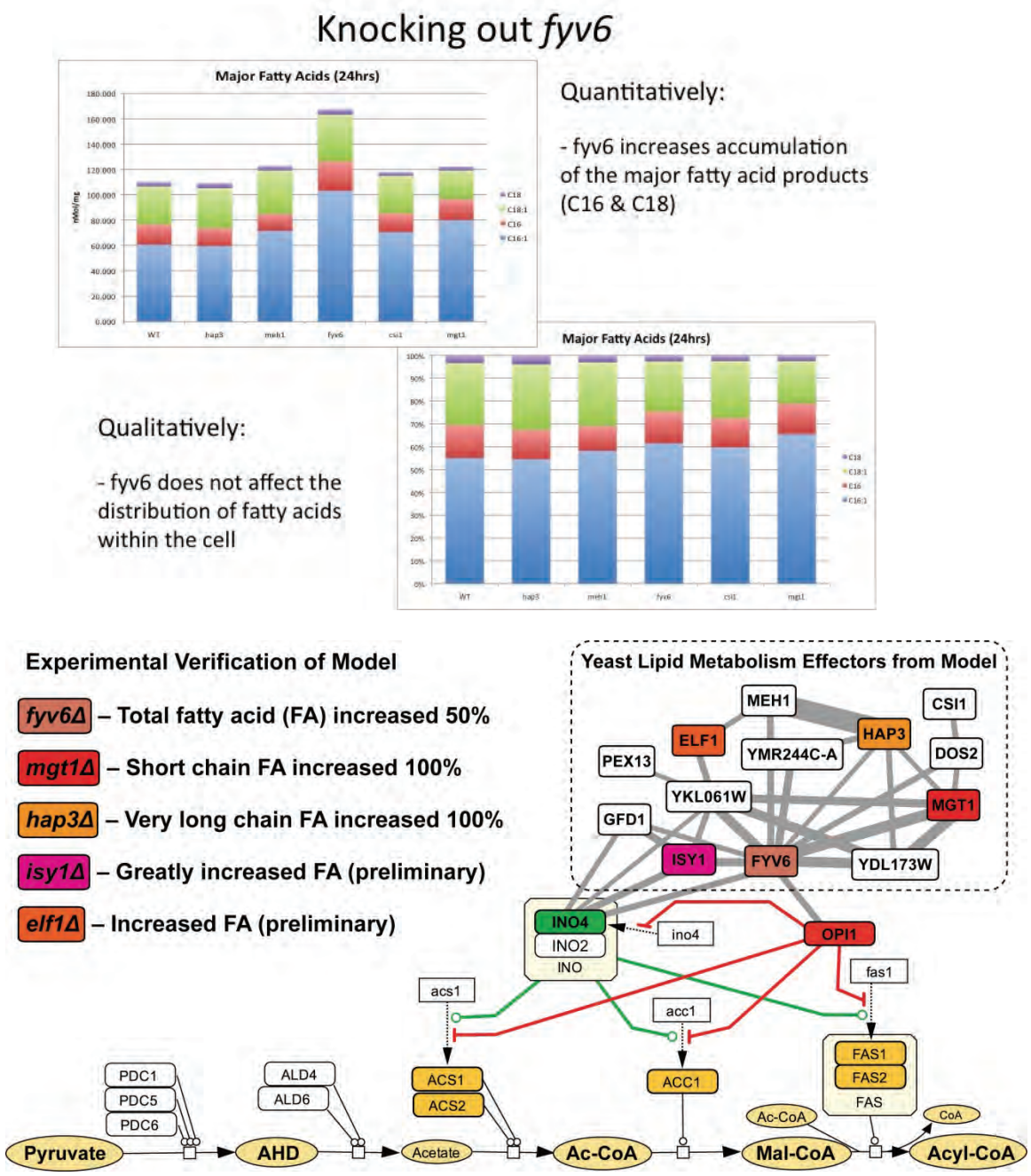
Fundamental Barriers and Methodologies

Meaningful data integration across heterogeneous data sources is confounded because the reliability of data sources and types is difficult to evaluate. Eukaryotic organisms such as yeast have multiple layers of regulatory and metabolic complexity, which prove a challenge for analysis. Surprisingly, much pathway information and gene functional annotation for yeast is still unknown.

Achievements

- We have established a direct pipeline to public transcriptomics data that enables the evaluation of extant experimental data in the context of experimental data and metadata. The extant transcriptomic data has been curated for quality, analyzed statistically, and used to create models of polyketide metabolism and its regulation. In this process, all extant transcriptomic data for yeast in the public database ArrayExpress was obtained and checked for replicate quality. Data from all samples with replicates over 95% similar and with adequate metadata was co-normalized. From this data, a global matrix was made of gene-pair-wise Pearson's correlations.
- The data has been statistically analyzed in combination with known fatty acid biosynthesis genes to identify genes that are likely to affect yeast lipid metabolism. This new model contains 15 yeast genes previously not known to be involved in lipid metabolism. Using a detailed yeast fatty acid/polyketide synthesis pathway, together with new genes identified via the global transcriptomics analysis model, we have generated a core model of the yeast polyketide metabolic and regulatory network. Such a model will be critical for optimal carboxylic acids and pyrone accumulation in yeast.
- We have focused on experimental analysis and verification of the 15 yeast genes that are implicated in our model as being involved in in regulating fatty acid/polyketide profile and content. Several gene-predictions of our new model have been verified by testing them experimentally in yeast knockout lines. These yeast strains have been tested for growth and fatty acid accumulation level and the results are consistent with our current model. The genes predicted by our model to be involved in lipid metabolism in yeast are being analyzed by examining strains of yeast that are knockouts of each gene, and conducting a combined growth analysis, live-cell staining, and GC-MS analysis of fatty acid from these mutant strains. To date, three novel genes from our model have been shown to significantly alter fatty acid levels or chain lengths. These are as follows.
- FYV is assigned as a protein of unknown function, and from it's sequence has been proposed to regulate double-strand break repair. The FYV6 mutant induces a 50% increase in total fatty acid ("*Knocking out fvy6*" figure).
- MGT1 is reported to be a DNA repair methyltransferase involved in protection against DNA alkylation damage. The MGT1 mutant induces a 100% increase in those fatty acids of 14-carbons and less.
- HAP3 is a considered transcriptional activator and global regulator of respiratory gene expression. The HAP3 mutant induces a 100% increase in fatty acid of 20-carbons and greater.
- Live-cell staining with Nile Red of the ISY1 and ELF1 mutants at multiple stages during their growth indicates that fatty acid levels are significantly increased; further analysis is on-going.

- The overall model, overlaid with the experimental verification to date, is shown below ("Model verification" figure)



Other Relevant Work

We have expanded our web-based database (MetNetOnLine) to explore and evaluate metabolic and signaling pathways in yeast. This structure will facilitate extraction of relevant network pieces from the model organisms and strains.

We have created a tool to process massive sets of multidimensional data to create clusters of functionally-, metabolically-, or expression-related genes on the fly.

Plans for the Next Year

The first three years provided the basis for a model of metabolic metabolism and signaling related to carboxylic acid and pyrone optimization and tools to maintain and access this model. In year 4, we have expanded our current model and evaluated new experimental data to verify the expanded model. In year 5, we will design and conduct targeted transcriptomics studies (T2 project 3b) to iteratively test the model of yeast lipid metabolism and its control.
In addition, we hope to use a yeast flux approach (T2 project 4b) to iteratively test the model of yeast lipid metabolism and its control. We will also leverage this experimental data, and those in other yeast-related Thrust 2 projects, to provide and integrate new information into the model. Thus, targeted transcriptomics studies, flux studies, and the network information-genetic algorithm approach will be used to expand and enhance the model. The results of this research will provide key information to empower yeast strain selection and development.

Expected Milestones and Deliverables

- Develop a combined network information-genetic algorithm approach. The algorithm will provide novel methods to evaluate 'omics data and metabolic flux data in the context of the metabolic model.
- Use the network approach to identify new edges and nodes in the network that may contribute to flux.
- Implement a data service solution to facilitate yeast metabolism and signaling data integration, access, and to enable network analysis and manipulation.
- Design and conduct targeted transcriptomics studies to iteratively test the model of yeast lipid metabolism.
- Integrate information based on targeted transcriptomics studies and network information-genetic algorithm approach to expand and enhance model. Use this data to provide information to empower yeast strain selection and development.

Member Company Benefits

The yeast network database and model would provide an excellent tool for industry researchers in their analysis of factors that contribute to composition in relation to polyketide (acetate-based) test beds. Furthermore, we have identifies specific genes that impact lipid/polyketide composition in yeast. The genes identified in our analyses will facilitate systematic manipulation of flux and yield to target carbon flow to the desired polyketide compounds.

The software developed can be applied to analysis of targeted manipulation of a wide range of compounds.

Commercialization / Technology Transfer

A provisional patent (no. 61/446.469) has been filed (based on an associated NSF-funded project) for a gene that controls accumulation of lipids, starches and oils.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T2.6A** – Beta-Oxidation Pathway Reversal in *E. coli*

Thrust: Thrust 2 - Microbial Metabolic Engineering

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Ramon Gonzalez	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Ramon Gonzalez, Rice University
Other Faculty: NA
Graduate Students: Maria Rodriguez-Moya, Seokjung Cheong, Rice University
Post-doctoral Scholars: James Clomburg, Jacob Vick, Elliot Miller, Rice University

Statement of Project Goals

This project aims to reconstruct a functional reversal of the β-oxidation cycle as a platform for the synthesis of functionalized carboxylic acids.

Project's Role in Center's Strategic Plan

The results from this project will directly contribute to the carboxylic acids test bed.

Fundamental Barriers and Methodologies

This project could be limited, in general, by the ability to establish an effective reversal of the β-oxidation cycle with functionalized primers.
Engineering components of the β-oxidation cycle reversal with novel properties will help in overcoming the above barriers.

Achievements

Reconstruction and functional characterization of a one-turn reversal of the β-oxidation cycle

We have recently developed *E. coli* strains capable of producing n-alcohols and carboxylic acids from glucose via a reversal of the β-oxidation cycle for fatty acid degradation (Nature 476:355-359, 2011). Four regulatory proteins were engineered to ensure that β-oxidation enzymes were expressed under the following conditions (manipulated regulator in parenthesis): i) absence of fatty acids, which are the natural substrates of and induce the β-oxidation pathway (non-functional FadR and constitutive AtoC), ii) use of glucose as carbon source, which represses the β-oxidation operons (cAMP-independent CRP mutant), and iii) use of anaerobic/microaerobic conditions (ArcA deletion). While the engineered strains produced n-alcohols and carboxylic acids, the minimum set of enzymes required to operate a functional reversal of the β-oxidation cycle were not identified, in part due to the use of the aforementioned mutations in global regulators.

Fig. 1A shows the proposed sequence of reactions and enzymes that mediate a one-turn reversal of the β-oxidation cycle using n-butanol synthesis as a proxy for *in vivo* functionality of the pathway. Enzymes are grouped in three metabolic modules. Module I "primes" the cycle by condensing two

molecules of acetyl-CoA (one acting as a primer and the other as 2-C donor) to form acetoacetyl-CoA, a reaction catalyzed by a short-chain thiolase. Module II encompasses three reactions: reduction of acetoacetyl-CoA to hydroxybutyryl-CoA (hydroxyacyl-CoA dehydrogenase), dehydration of hydroxybutyryl-CoA to crotonyl-CoA (enoyl-CoA hydratase), and reduction of crotonyl-CoA to butyryl-CoA (enoyl-CoA dehydrogenase/acyl-CoA dehydrogenase). Finally, Module III acts as a termination pathway, which in the case of n-butanol synthesis is composed of an acyl-CoA reductase (reduces butyryl-CoA to butyraldehyde) and an alcohol/aldehyde dehydrogenase (reduces butyraldehyde to n-butanol).

Once each of the aforementioned modules of the β-oxidation reversal is established, products with different functionalities can be synthesized by manipulating individual modules. For example, if a Module I thiolase capable of using succinyl-CoA as a primer is combined with a Module III thioesterase with high specificity for adipyl-CoA, the synthesis of adipate (a dicarboxylic acid) can be achieved (Fig. 1B).

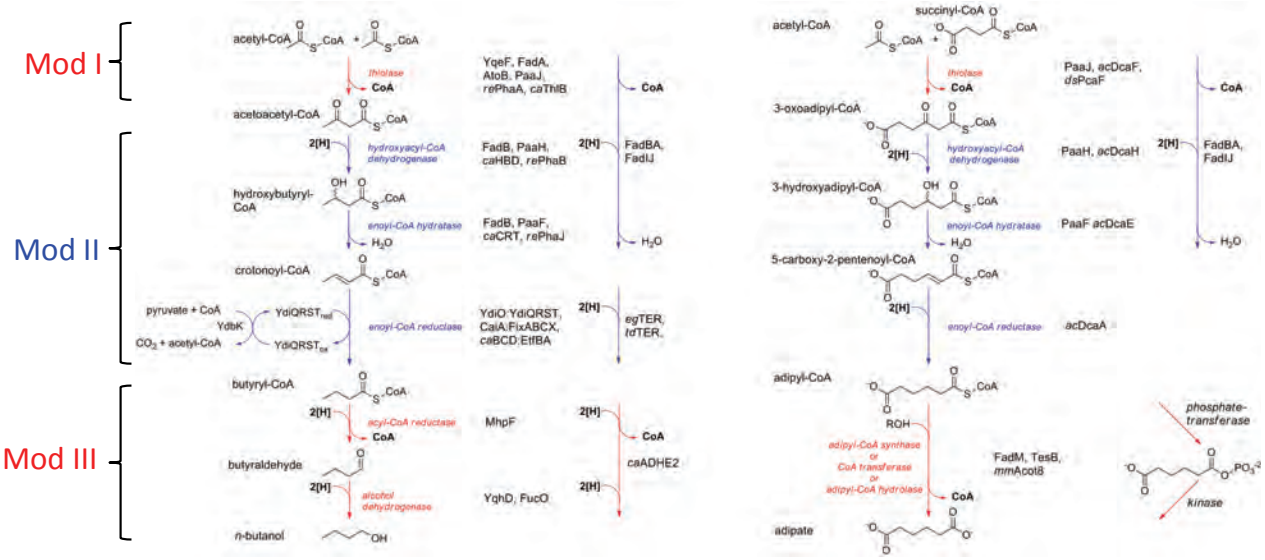


Fig. 1: One-turn reversal of the β-oxidation cycle with acetyl-CoA as the priming molecule and acyl-CoA reductase & alcohol/aldehyde dehydrogenase as termination enzymes (left) or succinyl-CoA as the priming molecule and thioesterase as the termination enzyme (right). Depending on the priming step (Module I) and termination pathway (Module III), the one-turn reversal can support the synthesis of n-butanol (left) or adipic acid (right).

Two primary tasks have been undertaken to re-construct the one-turn reversals of the β-oxidation cycle shown in Fig. 1 without engineering global regulators. First, the proposed gene products were purified and characterized *in vitro* for their designated activities. Second, a functional pathway is being assembled using the proposed genes from *E. coli* or other organisms. To this end, a series of plasmids were constructed to express genes hypothesized to have involvement in the one-turn reversal of the β-oxidation cycle with acetyl-CoA as the priming molecule and acyl-CoA reductase & alcohol/aldehyde dehydrogenase as termination enzymes, thus using n-butanol as the model product. These constructs were developed utilizing medium-copy (pTrcHis2A, pBR322-derived, oriR pMB1, *lacI*^{q}, *bla*) and low-copy (pZS, oriR pSC101*, tetR, cat, contains $P_{LtetO-1}$) vectors with different origins of replication and antibiotic markers to ensure their compatibility within the same bacterial strain. Genes identified as having involvement during the functional

reversal of the beta oxidation cycle (i.e. *yqeF/atoB, fadA, fadB, ydiO-ydiQRST, ydbK, mhpF, fucO*) were cloned into pTH and pZS constructs with the initial goal of expressing the priming and termination steps (thiolase and acyl-CoA reductase & aldehyde/alcohol dehydrogenase steps) in medium copy vectors and the central steps of the pathway (hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase, enoyl-CoA dehydrogenase/acyl-CoA dehydrogenase) in the low copy vector. In addition to this strategy, several vectors were constructed with steps in the pathway expressed from both pTH and pZS series vectors individually and in varying combinations in an attempt to provide a complete set of constructs to assess the required genes for a reconstruction of a functional β-oxidation cycle reversal.

Utilizing these initial constructs, as well as the complete set of cloned individual genes encoding Histidine-tagged proteins based on the genomic sequence data of *E. coli* K-12 (ASKA Library; National BioResource Project; DNA Research 12:291-299, 2005), a preliminary functional characterization of some of the proteins was undertaken with substrates expected with acetyl-CoA as the priming molecule and acyl-CoA reductase & alcohol/aldehyde dehydrogenase as termination enzymes (Fig. 1A). This included relevant enzyme assays with purified His-tag proteins in addition to assays with crude cell extract of additional constructs to ensure proper expression and functionally. For purified enzymes, SDS-PAGE was run to ensure sufficient purification and yield of the target proteins.

As seen in Fig. 2 purified AtoB exhibited significant thiolase activity in the catabolic direction (11 μmol/mg protein/min). YqeF, however, did not exhibit catabolic thiolase activity. We are currently implementing an assay in the synthetic direction to assess the catalytic efficiency of these thiolases in the functional reversal of the β-oxidation cycle. Purified FadB did not display any significant hydroxyacyl-CoA dehydrogenase activity. However, when the crude extract of wild-type cells expressing pTHfadBA was assayed, an activity of 4.1 μmol/mg protein/min was measured (Fig. 2). These findings indicate that the assembly of the FadBA complex may be required for proper function of FadB for the hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase reactions.

FucO, which has not been previously demonstrated to possess n-butanol dehydrogenase activity, exhibits significant activity for the reduction of butyraldehyde to n-butanol with NADH as a co-factor (5.08 μmol/mg protein/min), while no measurable butyraldehyde dehydrogenase activity was detected (Fig. 2). In addition, assays with purified MhpF gave an initial indication that this protein can be utilized for the reduction of butyryl-CoA to butyraldehyde as a small butyraldehyde dehydrogenase activity was measured with NADH as a co-factor (0.03 μmol/mg protein/min, data not shown). Testing of other purified proteins and constructs is currently underway and will lead to additional identification of proteins capable of catalyzing the essential steps of the pathway with either acetyl-CoA or succinyl-CoA as the primer molecule, and hence reconstruction of pathways for the synthesis of n-butanol and adipic acid.

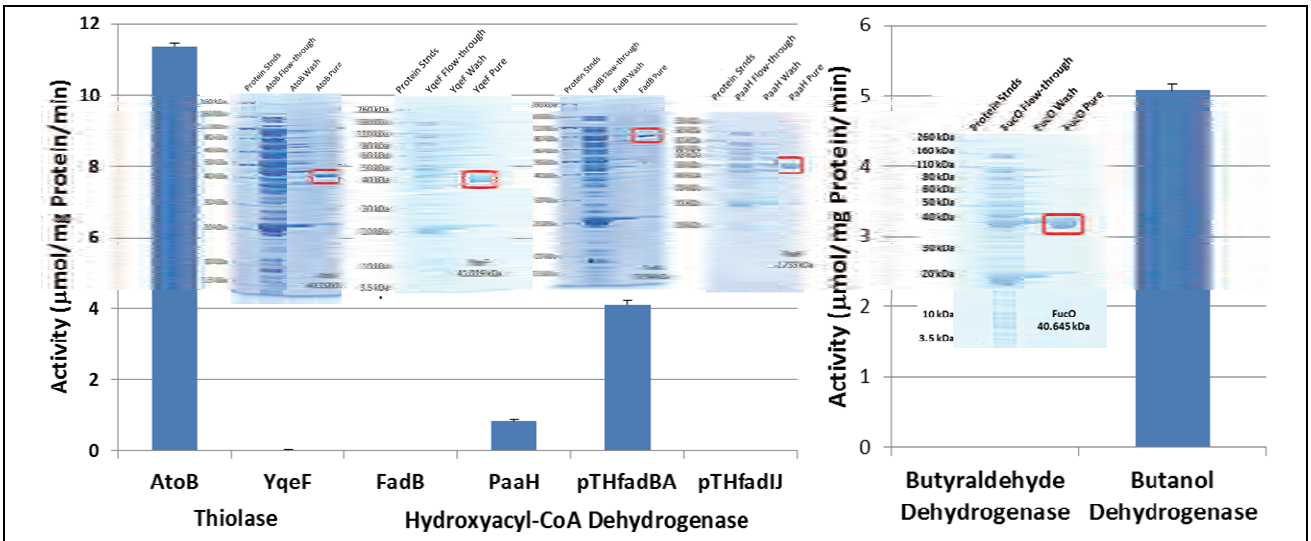


Fig. 2: Measured Module I (thiolase, left panel), Module II (hydroxyacyl-CoA dehydrogenase, left panel) and Module III (butyraldehyde/butanol dehydrogenase with FucO, right panel) activities with purified proteins and crude cell extract of cells expressing constructs (i.e. pTH"gene(s)"). SDS-PAGE gels of the purified proteins shown as insets: AtoB, YqeF, FadB and PaaH (left panel) and FucO (right panel).

In addition to providing an initial functional characterization of the proteins involved in a one-turn reversal of the β-oxidation cycle, these experiments also demonstrated several possible issues with the development of the most efficient set of constructs for product formation. Issues such as the requirement of FadA expression for a functional FadB, as well as the requirement for "auxiliary" proteins (YdiQ, YdiR, YdiS, YdiT and YdbK) for YdiO function and ferredoxin reduction underlie some of the complexities with the reconstruction of a functional pathway. Classic pathway engineering limitations such as proper expression levels of enzymes involved for maximal pathway flux and kinetic and enzymatic properties of the chosen proteins are of central importance and the ability to construct and test numerous combinations of proteins and expression levels is critical for the reconstruction efforts. Vector systems like the combination of pZS and pTH have some limitations as the number of restriction enzyme sites available limits the number of genes that can be incorporated. As a result, as the vectors grow in size, so does the potential for having to make additional silent mutations in the vector. Likewise, if and when it is found that it is necessary to replace a gene that was cloned into the vector in an early step, the whole pathway construction must be restarted to accommodate the change. A time intensive process that has a potential for additional mutations as every step requires PCR.

In order to address the above limitations, we are re-constructing the pathway using the recently reported set of optimized compatible BioBricks (Applied Microbiology and Biotechnology 92:1275-1286, 2011). The BioBrick paradigm allows for the quick exchange of specific parts with less chance of unexpected mutations as any gene, following an initial cloning into a BioBrick vector, is moved by sub-cloning. In addition, this set of BioBricks offers four different major host vectors each with a different combination of antibiotics, origins of replication (and resulting copy numbers). Since the process is built with modules, whole pathways can quickly be sub-cloned with a single digest and ligation from any one of these vectors to another vector without concern for what restriction enzymes are available in the destination vector. Likewise, as all the intermediate pathways are modular, swapping of specific genes is achieved quickly. Finally, the set of

BioBricks to be used offers additional options for control of expression levels by using different promoters (pT7, ptet and pBAD) that are also easily swapped for a gene once it is cloned into a BioBrick vector.

The initial design considers the expression of Modules I and III from a medium copy plasmid while a lower copy vector is reserved for the genes of Module II (Table 1). Based on the previous work, for the case of n-butanol as model product, Module I would be either *yqeF* or *atoB* while Module III will consist of *mhpF* and *fucO*. This combination of Module I and III would be assembled separately as pUCBB-yqeF or pUCBB-atoB for Module I options and Module III as pUCBB-mhpF-fucO. These would then be combined into the lower copy plasmid pCDFBB-yqeF-mhpF-fucO or pCDFBB-atoB-mhpF-fucO. Likewise, pUCBB-fadB and pUCBB-ydiO would be combined on the low copy plasmid as pACBB-fadB-ydiO. Lastly, *E. coli* strains would be co-transformed with both vectors and characterized for their ability to produce *n*-butanol.

While this "base" approach maybe viable, so far enzyme characterization experiments have identified some necessary modifications. For example, FadB and FadA most likely need to be co-expressed in order to realize *fadB*-encoded activities. In order to address this, the *fadBA* operon will be expressed as its native combination of genes and ribosome binding sites. To facilitate this, a new plasmid, pUCBB-noR-eGFP has been built that is compatible with the BioBricks system. pUCBB-noR-eGFP has had the optimized RBS (*aggagg*) removed allowing translation to be controlled by the native RBSs found in the *fadBA* operon. This requires the construction of pACBB-ydiO-noR-fadBA and pCDFBB-atoB-mhpF-fucO. It should be noted that FadA does not act as a thiolase for two acetyl-CoA's and so AtoB must be included, but a potential problem arises as FadA does accept longer acyl chains, potentially resulting in a loss of *n*-butanol production. A potential solution is to express an inactive FadA; a FadA that is incapable of catalyzing the thiolase reaction but still capable of forming a complex with FadB. And as *in vitro* assays continue, *fadBA* could be replaced with the functionally homologous *fadIJ* or *PaaJ*.

Table 1: Different approaches to reconstruct a one-turn reversal of the β-oxidation cycle using n-butanol as model product. Module I and III (see Fig. 1) are combined and expressed from the medium copy plasmid pCDFBB. Module II (see Fig. 1) is expressed from low-copy plasmid pACBB. The "base" approach has 2 potential vector pairings while the FadBA-based approach has 8 potential pairings.

Approach	**Module II Low copy - pACBB**		**Module I & III Med copy - pCDFBB**
Base	fadB-ydiO	+	yqeF-mhpF-fucO atoB-mhpF-fucO
FadBA-based approach	fadBA-ydiO-ydiQRST-ydbK fadBA-caiA-fixABCX-ydbK fadBA-*td*TER fadBA-*eg*TER	+	atoB-mhpF-fucO yqeF-mhpF-fucO

While YdiO is the predicted enoyl-CoA reductase, unlike the aforementioned redox catalyzing enzymes, it does not utilize NAD(P)H. YdiO requires the ferredoxins and flavoproteins encoded by the *ydiQRST* operon in order to properly function. Additionally, in order to produce reduced ferredoxin, pyruvate:flavodoxin/pyruvate:ferredoxin oxidoreductase (YdbK) must also be included. This has added advantages, as a direct coupling between generation and consumption of reducing equivalents (reduced ferredoxin) can be established: i.e. YdbK-YdiO coupling. Thus, a pUCBB-noR-ydiQRST-ydbK has been constructed as well to facilitate YdiO activity. This pUCBB-noR-ydiQRST-ydbK can be left on the high copy pUCBB, easily moved to pBBRBB, or added to either the Module I and III genes on pCDFBB or Module II genes on pACBB. CaiA-FixABCX will be evaluated as an alternative to YdiO-YdiQRST. A less complicated option maybe to replace the YdiO with the NAD(P)H dependent *trans*-enoyl-CoA reductase (TER) from either *Euglena gracilis* (*eg*TER) or *Treponema denticola* (*td*TER). Using a TER would also decrease the metabolic burden on the cell due to not having to produce all the "helper" proteins for YdiO or CaiA.

Use of reconstructed one-turn reversal of the β-oxidation cycle for the synthesis of adipic acid

In addition to the reconstruction and functional characterization of a one-turn reversal of the β-oxidation cycle with acetyl-CoA as the priming molecule and acyl-CoA reductase & alcohol/aldehyde dehydrogenase as termination enzymes (leading to n-butanol synthesis, Fig. 1A), the above detailed BioBricks constructs will also be functionally assessed for adipic acid production via succinyl-CoA as the priming molecule and thioesterase as termination enzyme (Fig. 1B). However, initial tests have also shown that *E. coli* is not able to metabolize adipic acid as a carbon source even with an active β-oxidation cycle. As seen in Fig. 3, wild-type *E. coli* K12 strain MG1655 exhibits minimal growth on either caproic acid or adipic acid. However, when the fadR atoC(con) genotype is present, the cells are able to utilize caproic acid as a carbon source (indicating an active β-oxidation cycle) but still do not possess the ability to grow with adipic acid as a carbon source. This result indicates that i) *E. coli* lacks a sufficient transport mechanism for adipic acid and/or ii) enzymes active with a de-regulated β-oxidation cycle do not act on the functionalized acyl-CoA intermediates involved in the metabolism of adipic acid (reversal of Fig. 1B). While both scenarios are of critical importance for the use of the β-oxidation cycle reversal with succinyl-CoA as the priming molecule, the latter would require the identification of additional proteins capable of acting on these intermediates different from those determined during the characterization of a one-turn reversal for n-butanol production.

Based on the above findings, the reversal of the β-oxidation with succinyl-CoA as a primer may require the use of alternative enzymes as either direct replacements for enzymes involved in n-butanol production or require the identification of suitable transport proteins for the export/import of adipic acid. While *E. coli* has not been shown to metabolize adipic acid, several organisms have been documented to harbor the genes encoding a medium chain dicarboxylic acid β-oxidation pathway including *Acinetobacter* sp. ADP1 (Applied and Environmental Microbiology 67:4817-54827, 2001) and *Rhodopseudomonas palustris* (Microbiology 151:727-736, 2005). More specifically to the case of adipic acid, *Acinetobacter* sp. ADP1, has been documented to degrade adipic acid (Applied and Environmental Microbiology 67:4817-54827, 2001) and the sequence information for some of the putative genes believed to be involved in this process are available (Fig. 1B), including a possible transporter protein (*dcaK*) and porin (*dcaP*). In addition to these genes, the synthesis of adipate will also require the activity of termination enzyme(s) capable of

converting adipyl-CoA to adipate. For initial testing, the termination enzymes will include the *E. coli* thioesterases *tesB* and *fadM*, as well as the mouse acyl-CoA thioesterase (*Acot8*), which has been shown to hydrolyze CoA esters of dicarboxylic acids with high activity (Journal of Biological Chemistry 280:38125-38132, 2005).

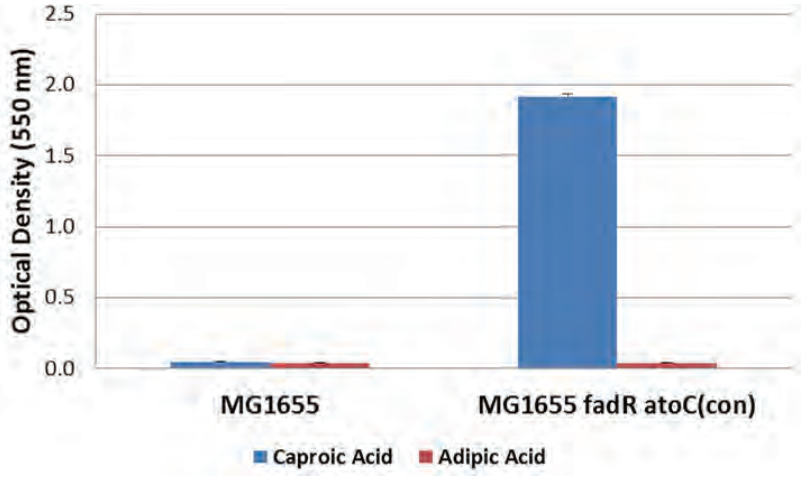


Fig. 3: Cell growth of MG1655 and MG1655 fadR atoC(con) on caproic acid and adipic acid as the sole carbon source.

As a first approach to assess the adipic acid transport and degradation limitations of *E. coli* and the compatibility of theoretical replacement genes from other organisms, the adipic acid transport and degradation pathway from *Acinetobacter* sp. ADP1 are being constructed in separate constructs and expressed in *E. coli* to determine the genes required to enable adipic acid degradation. Once the set of enzymes required for the degradation pathway are determined, these specific proteins can be characterized for their ability to function in the synthetic direction, enabling their use in the functional reversal of the β-oxidation cycle with succinyl-CoA as the priming molecule.

Initial work in this area led to the finding that purified PaaH displayed a significant hydroxyacyl-CoA dehydrogenase with acetoacetyl-CoA as a substrate. This is of special interest for the one turn reversal of the β-oxidation cycle with succinyl-CoA as the priming molecule, as PaaH is also postulated to catalyze the reduction of 3-oxoadipyl-CoA to 3-hydroxyadipyl-CoA (Fig. 1B). Additional assays with 3-oxadipyl-CoA as the substrate will confirm if this protein can catalyze the hydroxyacyl-CoA dehydrogenase step with either acetyl-CoA or succinyl-CoA as the priming molecule during the first turn of the β-oxidation cycle reversal.

Quantitative assessment of the biosynthetic capabilities of a functional reversal of the β-oxidation cycle

We have assessed the metabolic capabilities of a functional reversal of the β-oxidation cycle by using a genome-scale model (Feist et al., 2007. Molecular Systems Biology 3:121) and metabolic optimization (Flux Balance Analysis). We modeled the anaerobic synthesis of n-alcohols, alkanes and fatty acids of different chain lengths (from C3 to C16). Fig. 4 shows model predictions for the synthesis of n-butanol (C4 alcohol) via a functional reversal of the β-oxidation cycle. The results are shown for both wild-type *E. coli* and an engineered strain where pathways for competing

fermentation products were eliminated (namely ethanol, acetate, lactate, and succinate). It is noteworthy that elimination of competing fermentation products led to a coupling between target product synthesis and cell growth due to the redox-balanced and ATP-generating nature of the engineered pathway. Fig. 4 also shows the synthesis of n-butanol using the fatty acid biosynthesis pathway, which is less efficient than the functional reversal of the β-oxidation cycle, presumably due to its lower ATP yield.

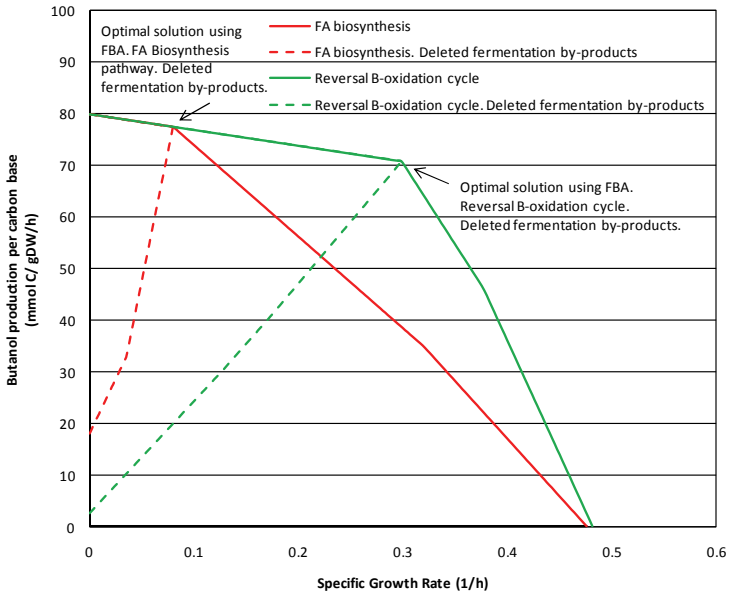


Fig. 4. Analysis of n-butanol production in *E. coli* using flux balance analysis. Two different metabolic platforms for the synthesis of acyl-CoA intermediates from acetyl-CoA were analyzed: 1) reversal of the β-oxidation cycle (green) and 2) fatty acid biosynthesis pathway (red). Solid lines represent the base case of strain with all the fermentative pathways active, while broken lines represent a strain in which pathways leading to the synthesis of fermentation products ethanol, acetate, lactate, and succinate were eliminated.

Table 2 presents a summary of key findings when the synthesis of n-butanol and n-hexadecanol was simulated in the aforementioned engineered strains. Although the yield of n-butanol is slightly higher when the fatty acid biosynthesis pathway is used, the specific growth rate and the volumetric production of butanol were higher when the reversal β-oxidation cycle was used (275% and 14%, respectively). Moreover, deletion of fermentation by-products impaired the fermentative production of longer chain alcohols (n-hexanol and higher) when the FA biosynthesis pathway is used. However, synthesis of longer-chain alcohols via the reversal of the β-oxidation cycle was predicted to be as efficient as the synthesis of n-butanol. Similar results were obtained when the synthesis of alkanes and fatty acids was evaluated, demonstrating the efficient nature of the reversal β-oxidation cycle for the fermentative synthesis of n-alcohols, fatty acids, and alkanes.

Table 2. Optimal synthesis of n-butanol and n-hexadecanol by a strain with deletions in pathways leading to the synthesis of fermentation products ethanol, acetate, lactate, and succinate. Two different metabolic platforms for the synthesis of acyl-CoA intermediates from acetyl-CoA were analyzed: 1) reversal of the β-oxidation cycle and 2) fatty acid biosynthesis pathway. (NS): No solution.

	Coupling biomass to product	Specific growth rate (1/h)	Production flux per carbon base (mmol C/gDW/h)	Volumetric production (mmol C/L/h)	Yield (mol C /mol C)
n-butanol – FA biosynthesis pathway	Yes	0.08	77.53	83.99	0.65
n-butanol – Reversal β-oxidation cycle	Yes	0.30	70.82	95.50	0.59
n-hexadecanol – FA biosynthesis pathway	(NS)	(NS)	(NS)	(NS)	(NS)
n-hexadecanol – Reversal β-oxidation cycle	Yes	0.26	71.99	93.46	0.60

Other Relevant Work

Plans for the Next Year

A complete re-construction and functional characterization of the β-oxidation cycle will be conducted.

Termination, elongation and priming modules of the reversal of the β-oxidation cycle will be modified to achieve the synthesis of functionalized carboxylic acids and alcohols.

Expected Milestones and Deliverables

For next year, we expect to:

1. The reconstruction and functional characterization of the one-turn reversal of the β-oxidation cycle will be completed.
2. Synthesis of adipic acid through a one-turn reversal of the β-oxidation cycle will be achieved.

Member Company Benefits

The implementation of a modular pathway for the efficient synthesis of functionalized carboxylic acids and alcohols is expected to generate significant intellectual property, which in turn will benefit member companies.

Commercialization / Technology Transfer

Opportunities for technology transfer and commercialization are expected from the work.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.2 -** Selective Dehydration of Model Compounds

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Brent Shanks	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Brent Shanks, Iowa State University

Collaborator: James Dumesic, University of Wisconsin-Madison

Graduate Students: Michael Nolan and Tianfu Wang, Iowa State University, Yomaira J. Pagan-Torres, University of Wisconsin-Madison

Statement of Project Goals

Biorenewable feedstocks have excess oxygen relative to the amount typically present in industrial chemicals. Dehydration is an important reaction for the removal of oxygen, but limited work has been performed on selective dehydration in the presence of additional functionality in the reactant. An important goal in developing a catalytic "tool chest" for biorenewable chemicals will be demonstration of effective selective dehydration catalysts.

Project's Role in Center's Strategic Plan

Selective dehydration in general will be an important chemical catalyst capability in the center. In particular, selective dehydration catalysts will be necessary for successful development of bifunctional chemicals such as 1,6-hexanediol or 5-hydroxymethyl furfural (HMF). Due to the presence of excess functional groups (such as carbonyl and/or hydroxyl groups) in biorenewable feedstocks as well as the use of water as a reaction media, the developing novel catalyst system will be required.

Fundamental Barriers and Methodologies

Our work in selective dehydration is addressing the fundamental question of whether catalytic dehydration can be selectively performed on a molecule with multiple oxygen-containing functional groups as would be required for converting carbohydrate-derived molecules. Two separate challenges are being explored in this work; 1) the selectivity barrier in dehydration of polyols that allows selective formation of linear species relative to ring-shaped species, and 2) the selectivity barrier encountered in glucose dehydration in which the reaction is selective to HMF.

Acid-catalyzed dehydration of 1,2,6-hexanetriol favors selectivity to pyran ring products over the desired linear products. New understanding over the last year has found that the main selectivity driver appears to be the activated species in the dehydration reaction, and manipulation of reaction kinetics could enhance selectivity to linear species.

Glucose is more difficult to convert selectively to HMF than fructose given the lower reactivity

of the pyranose structure. We are examining the combined use of Lewis and Brønsted acidity to allow the conversion of glucose to proceed through a tandem reaction pathway involving isomerization of glucose to fructose and subsequent dehydration of fructose to form HMF as catalyzed by Lewis and Brønsted acids, respectively. To minimize the HMF degradation in the reactive aqueous phase, a biphasic reactor system using an extracting layer in contact with an aqueous phase was used. Further work is required to understand the nature of interaction between Lewis acids and the carbonyl groups in water to provide more information for rational design of an effective catalyst system.

Achievements

Selective dehydration of 1,2,6-hexanetriol

Investigations into the dehydration reaction pathway for 1,2,6-hexanetriol included carrying out the dehydration reaction with a range of catalysts with varying acid strength and substituting observed products in place of 1,2,6-hexanetriol. The primary observation across all of the tests was selectivity to 1,6-dioxygenates and pyran ring products, with no observed selectivity to 1,2-dioxygenates and trace (~0.1%) selectivity to 1,5 dioxygenates, which suggested the dehydration reaction proceeds nearly exclusively via protonation of the 2-position alcohol in 1,2,6-hexanetriol, and all further selectivity is driven by how the molecule stabilizes the intermediate cation. An example of the reaction intermediate and its role in selectivity is given in Figure 1.

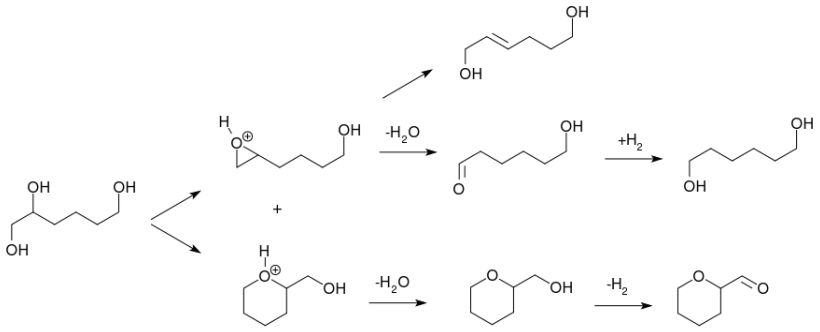
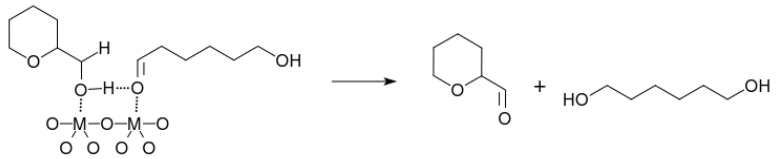


Figure 1: Proposed dehydration pathways for 1,2,6-hexanetriol via cation intermediates

This fundamental selectivity was found to remain unchanged as a function of catalyst acid strength. Selectivity between pyran rings and linear molecules was found to be driven mainly as a function of temperature, which suggested a kinetic effect for selectivity, with the two intermediates in Figure 1 representing two different activation energies. Therefore, selectivity could be changed by modifying either the reaction temperature or the relative stability of each intermediate in the catalytic. A collaboration with the Neurock group, planned for the upcoming year, will examine this selectivity effect computationally.

Hydride transfer reactions were also observed over the acid catalysts, with the most interesting being the formation of 1,6-hexanediol and caprolactone. GC-MS found that 6-hydroxyhexanal was formed as a major product when 1,2,6-hexanetriol dehydrated, and our working hypothesis is that Lewis acidity permits inter-molecular hydride transfer in the absence of metals. In the case of 1,6-hexanediol, as illustrated in Figure 2, 6-hydroxyhexanal (generated as shown in Figure 1) can abstract a hydrogen from an alcohol C-O bond if both molecules are present on the catalyst surface. This effect is observed in the reaction results as a stoichiometric balance of oxidized and reduced molecules, typically in the form of an oxidized pyran product appearing alongside 1,6-hexanediol.



Selective dehydration of glucose to HMF

We demonstrated the conversion of glucose to HMF in a biphasic reactor system in the presence of Lewis acid salts ($AlCl_3$, $SnCl_4$, VCl_3, $InCl_3$, $GaCl_3$, $LaCl_3$, $DyCl_3$ and $YbCl_3$). The biphasic reactor consisted of an aqueous layer saturated with NaCl and an extracting organic layer consisting of 2-*sec*-butylphenol (SBP). The combination of Lewis and Brønsted acidity could produce HMF by a combined isomerization/dehydration reaction of glucose to HMF.

It can be seen in Table 1, entries 1-2, that glucose was converted in the presence of HCl, without any other metal chloride catalyst; however, the HMF selectivity was only 30% at 91% conversion. In the absence of an isomerization catalyst, the formation of HMF may follow a mechanism, in which the open-chain form of glucose is dehydrated at the C-2 position, forming a carbocation which reacts with the hydroxyl group at C-5 position, forming tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)-2-furaldehyde followed by further dehydration to form HMF. The addition of a Lewis acid salt clearly led to a significant increase in HMF selectivity, as shown in Table 1, entries 3-10. The presence of $AlCl_3$ led to 68% HMF selectivity at 91% conversion. Moderate HMF selectivities were obtained using other metal chlorides such VCl_3, $InCl_3$, $GaCl_3$, $YbCl_3$, $DyCl_3$ and $LaCl_3$.

Table 1. Conversion of glucose to HMF in a biphasic system with SBP as extracting organic layer.[a]

Entry	Catalyst	Conversion [%]	Selectivity [%]	Time [min]
1	-	15	52	30
2	-	91	30	420
3	$AlCl_3$	91	67	40
4	$SnCl_4$	90	58	45
5	VCl_3	92	53	90
6	$GaCl_3$	90	50	120
7	$InCl_3$	86	52	150
8	$YbCl_3$	93	46	120
9	$DyCl_3$	93	41	160
10	$LaCl_3$	87	44	240

Table 2. Comparison between extracting organic layers for the conversion of glucose to HMF in a biphasic reaction system using $AlCl_3$ and HCl as catalysts.[a]

Extracting Organic Layer	Conversion [%]	Selectivity [%]	% HMF in Org	% HCl in Org
THF	80	71	93	30
SBP	88	70	97	0

[a] Reaction conditions: 5 wt% glucose in water saturated with NaCl, glucose to Lewis acid molar ratio of 67, pH = 2.5, reaction temperature of 443 K, organic to aqueous mass ratio of 2.

Using SBP as the extracting solvent led to improved HMF yields and enhanced partitioning of the HMF (Table 2) relative to THF. An additional important advantage with the SBP solvent was that none of the HCl was detected in the organic layer, in contrast to the system with THF in which 30% of the HCl was extracted into the organic layer.

To further demonstrate the effectiveness of the water-SBP biphasic system with $AlCl_3$ and HCl in the conversion of glucose to HMF, experiments were conducted to assess the extent to which the acidic aqueous layer could be recycled. For these experiments, four consecutive runs were carried out, each with a reaction time of 40 min. Between each run, the organic layer containing HMF was extracted, and fresh SBP and glucose were added to the acidic aqueous solution in the reactor. No significant decreases in conversion and selectivity were observed, as shown in Figure 1.

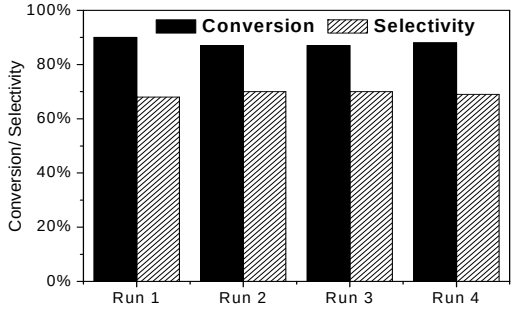


Figure 3. Conversion and selectivity for production of HMF from glucose in consecutive runs using the same acidic aqueous solution containing $AlCl_3$ and HCl as catalysts.

While a low solution pH is required for most conventional Lewis acid salts to maintain their Lewis acidity in the presence of water, we found that the conversion of glucose to HMF catalyzed by a series of water-compatible Lewis acids, the lanthanide metal salts ($LaCl_3$, $DyCl_3$ and $YbCl_3$), could function under wide range of pH values (1.5, 2.5, 3.5, 4.5 and 5.5). Operation at higher pH is quite desirable to minimize the use of strong mineral acids. Shown in Table 2 is the summary of the reaction results with water-compatible Lewis acids.

Table 3. Results of glucose conversion to HMF in a biphasic reactor.[a]

Entry	Catalyst	Catalyst concentration [mM]	Conditions	Conversion [mol%]	Selectivity [mol%]	Time [min]
1	N/A	N/A	pH=5.5	20	53	80
2	N/A	N/A	pH=5.5	45	53	240
3	$YbCl_3$	25	pH=1.5	84	40	40
4	$YbCl_3$	25	pH=2.5	88	48	80
5	$YbCl_3$	25	pH=3.5	88	46	80
6	$YbCl_3$	25	pH=4.5	87	46	80
7	$YbCl_3$	25	pH=5.5	88	45	80
8	$YbCl_3$	100	pH=5.5	91	46	40
9	$DyCl_3$	25	pH=5.5	89	44	120
10	$LaCl_3$	25	pH=5.5	86	46	200

[a] Reaction conditions: 5 wt% glucose in nanopure water saturated with NaCl, pH adjusted with HCl, reaction temperature of 443 K, organic/aqueous mass ratio= 2 with 2-sec-butyl-phenol used as the organic layer to extract HMF from the aqueous phase.

Other Relevant Work

Plans for the Next Year

A slate of acid-metal bifunctional catalysts has been prepared for the coming year, and will be used to study hydrogenolysis of 1,2,6-hexanetriol in the gas phase. Bifunctional catalysts will include platinum and palladium on metal oxides and zeolites for gas-phase testing, and will include metals and metal oxides supported on carbon for condensed-phase testing. A collaboration with Qiaohua Tang from the University of Virginia will be initiated to computationally simulate dehydration of 1,2,6-hexanetriol over acid catalysts, and to corroborate the reaction data results with the simulation. This will further elucidate the catalytic options available for selective dehydration of polyols. We will be examining translation of HMF catalyst technology to GlucanBio. While the preliminary results demonstrated the feasibility and potential of using the combination of Lewis/Brønsted acid catalysts, further work will be needed to better understand the mechanism of the catalytic process. Also, the development CNT-supported catalytic species with both Lewis and Brønsted acids characteristics will be explored.

Expected Milestones and Deliverables

A slate of acid-metal bifunctional catalysts will be characterized and tested in the gas phase, and bifunctionals on carbon supports will be characterized and tested in the liquid phase. Reactions will be carried out in reducing and non-reducing conditions to study both hydrogenolysis and hydride transfer for 1,2,6-hexanetriol. Testing will also be carried out with HMTHP (selective ring opening) and 1,6-hexanediol (selective dehydrogenation to 6-hydroxyhexanal, and upgrading to caprolactone). CNT-supported solid Lewis acid catalysts for glucose dehydration to HMF will be synthesized and tested by February, 2013. Publications submitted:

[1] Y. Pagan-Torres, T. Wang, J. Gallo, B. Shanks and J. Dumesic, Production of 5-Hydroxymethylfurfural from Glucose Using a Combination of Lewis and Brønsted Acid Catalysts in Water in a Biphasic Reactor with an Alkylphenol Solvent, submitted.

[2] T. Wang, Y. Pagan-Torres, E. Combs, J. Dumesic and B. Shanks, Water-compatible Lewis acid-catalyzed conversion of carbohydrates to HMF in a biphasic solvent system, submitted.

Member Company Benefits

A catalyst and reactor conditions that lead to high yields of HMTHP when coupled with selective ring opening to 1,6-hexanediol could be an attractive route to a valuable monomer species. Further understanding of hydride transfers could also broaden the array of attractive molecules that can be derived from dehydration reactions. The economic production of HMF is of importance since HMF could be converted to a rich array of chemical intermediates. The potential of economical HMF production will benefit a number of our member companies in diversifying their current feedstock supply to include biomass.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.3** – Deoxygenation of Fatty Acids

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Robert Davis	**02/17/12**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Robert Davis, University of Virginia.
Other Faculty: Keith Woo, Iowa State University; George Kraus, Iowa State University; Matthew Neurock, University of Virginia
Graduate Students: Jonathan Beasley and Sean Riley, Iowa State University; Ben Huang and Juan Alberto Lopez-Ruiz, University of Virginia

Statement of Project Goals

Develop an efficient and scalable preparation of alpha-olefins or other hydrocarbons from fatty acids using homogeneous or heterogeneous catalysis. Define the scope and limitations of the procedures.

Project's Role in Center's Strategic Plan

One of the integrative test beds in this Center involves the production of alkenes. Since fatty acids are readily produced by fermentation (Thrust 2), an efficient catalyst that converts fatty acids into alpha-olefins (plus CO and water) is needed.

Fundamental Barriers and Methodologies

The transformation of fatty acids into alpha-olefins has been studied very little compared to the conversion of fatty acids to saturated hydrocarbons. A major fraction of previous work in the area of deoxygenation of carboxylic acids uses palladium as the transition metal catalyst and also dihydrogen, H_2, to prevent the catalyst from rapidly deactivating. Unfortunately, alpha-olefins are readily hydrogenated into paraffins in the presence of H_2 and transition metal catalysts. Thus, the work in this project explores catalyst compositions and reaction conditions that allow for decarbonylation while minimizing the subsequent conversion of product olefins. An important undesirable side reaction is water gas shift, WGS, since it produces hydrogen that could saturate the product olefins. Also, the ketonization of the fatty acids can also occur on solid catalysts and may even be an intermediate step in the formation of alpha olefins from the carboxylic acids. However, we found that when catalyst supports that were effective at ketonization were tested, the catalysts were less effective than those prepared with inert carbon supports. Thus, we concluded that ketonization is not an important reaction in the overall route to olefins but is instead a side reaction that consumes the fatty acid.

Achievements

We have successfully identified a stable catalyst that produces α-olefins from carboxylic acids at

high selectivity, without the addition of small amounts of hydrogen to maintain activity. More specifically, we have tested the liquid-phase conversion of heptanoic acid to 1-hexene on solid catalysts in a fixed bed flow reactor. Supported platinum, 3 weight percent loading, on activated mesoporous carbon was highly selective towards the formation of 1-hexene, ~80%, and very stable for a period of 120 hours at 573 K and 37 bar. The figure and table below illustrate some representative results from reactivity studies.

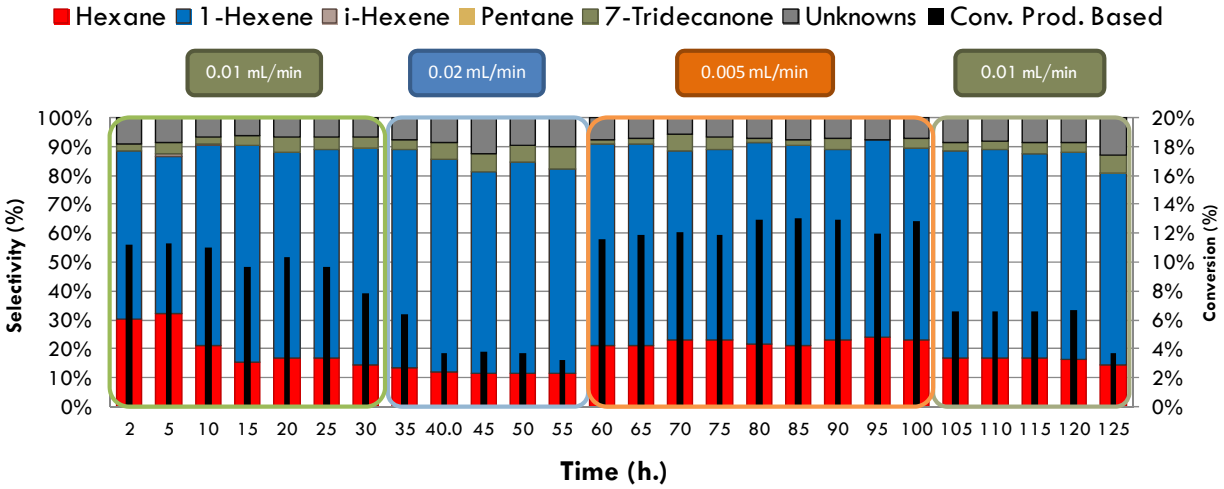


Figure 1. Effect of feed flowrate on performance of 3 wt% Pt/C. Conversion (black bars) on the right axis and selectivities (color bars) on the left axis. Reaction conditions: 573 K, 37 bar, and 0.25g of 3 wt% Pt/C.

Table 1. Effect of temperature and reduction temperature on catalyst activity. Reaction conditions: 0.01mL/min feed of heptanoic acid, 37 bar total pressure, and 0.25 g of 3 wt% Pt/C or Pd/C.

	Temp.	Disp.	TOF	Conversion	Liquid Product Selectivity (%)				$\frac{CO}{CO+CO_2}$
	K	%	s^{-1}	%	Hexane	Hexenes	7-Tridecanone	Unknowns	
3Pt873	573	13	0.016	7.2	20.1	76.1	0.8	3.0	59.4
3Pt773	593	13	0.046	22.7	13.4	82.3	1.9	2.4	67.5
	573		0.022	9.6	12.7	79.9	3.3	4.0	59.3
	553		0.007	3.3	10.4	77.6	9.0	2.9	74.2
3Pt673	593	25	0.019	17.5	11.2	85.9	0.7	2.2	71.7
	573		0.009	7.3	10.3	84.2	1.2	4.4	70.6
	553		0.006	4.9	9.4	82.7	4.8	3.2	54.5
3Pt623	593	37.5	0.046	19.9	13.8	80.8	3.3	2.0	63.9
	573		0.020	9.5	10.9	84.2	0.3	4.5	63.9
3Pd673	573	-	-	7.7	28.6	61.2	6.8	3.0	72.1
3Pd873	593	-	-	16.1	25.8	69.0	3.5	1.6	71.6
	573		-	7.4	24.1	67.9	5.5	2.4	73.7
	553		-	4.9	22.8	69.8	4.9	2.4	77.1

The results in Table 1 show the effectiveness of supported Pt for decarbonylation. In addition, preliminary experiments with supported Pd also showed that alpha-olefins can be produced from

fatty acids, although the selectivity to alpha-olefin from heptanoic acid appears to be lower than that from the Pt catalysts. Turnover frequencies for the Pd catalysts will be calculated after the dispersion has been determined. It should be noted that carbon was the chosen support since earlier work in the lab with oxide supports such as ceria, zirconia, and ceria-zirconia enhanced the formation of ketones instead of promoting the formation of olefins.

In summary, supported metal catalysts are being modified and characterized to understand the effects of composition, metal loading, particle size, and preparation conditions on the turnover frequency, product selectivity, and catalyst stability in fatty acid decarbonylation.

Plans for the Next Year

We are working to optimize the catalyst preparation conditions to improve reaction rate and selectivity towards the formation of alpha-olefins. Furthermore, we will study the effect of bimetallic catalysts on the overall catalyst performance and also we will perform mechanistic studies to unravel the key reaction steps that lead to the formation of alpha-olefins from carboxylic acids. We are also examining the performance of non-precious metal catalysts for the reaction.

Expected Milestones and Deliverables

The project will determine optimum rates and selectivities that can be used for process design calculations.

Member Company Benefits

Members will have access to results from experimental studies of selective decarbonylation reactions catalyzed by supported metal catalysts. The member companies will also have access to catalyst synthesis and characterization techniques for supported metal catalysts.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.5** – Ring Opening Reactions

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
James Dumesic	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: James Dumesic, University of Wisconsin-Madison

Faculty: Matthew Neurock, Robert J. Davis, University of Virginia; Abhaya K. Datye, University of New Mexico

Graduate Students and Staff: Mei Chia, Thomas Schwartz, University of Wisconsin-Madison; David Hibbitts, Qiaohua Tan, Mohammad Ali Haider, University of Virginia; Hien N. Pham, University of New Mexico

Statement of Project Goals

The overall goal of this work is to develop catalysts for the selective ring-opening and hydrogenation of heterocyclic compounds derived from biomass and to elucidate the factors that control selectivity in these reactions.

Project's Role in Center's Strategic Plan

The ability to perform selective ring-opening of heterocyclics over heterogeneous catalysts is essential for the deoxygenation of biomass-derived feedstocks to produce value-added chemicals. In this project, we examine the hydrogenolysis of C-O bonds of furans and pyrans, ring-opening and decarboxylation of pyrones, and the factors that affect product selectivities in these reactions. We also examine the inhibitive effect of small amounts of biogenic compounds on the catalytic activity of supported metal catalysts for the hydrogenation of pyrones, which is a critical factor in the upgrading of biologically-synthesized pyrones. This work is in line with the Center's strategic interests in selective ring-opening, hydrogenolysis, and upgrading of pyrones.

Fundamental Barriers and Methodologies

The main challenge for achieving selective ring-opening of furans and pyrans is to selectively cleave specific C-O bonds in these molecules without the formation of other by-products or further degradation of the desired product. Our initial studies revealed that a rhodium-rhenium catalyst supported on carbon displays both of these desired reactivity characteristics for the hydrogenolysis of tetrahydropyran-2-methanol and tetrahydrofurfuryl alcohol to their respective α,ω-diols. The focus of our subsequent work has been to obtain fundamental understanding of the nature of the active sites for this rhodium-rhenium catalyst using a combination of experimental data and quantum chemical calculations. As part of efforts in development of the Pyrone Testbed, our experimental work in the ring-opening of pyrones

demonstrated that the ring-opening and decarboxylation of certain pyrones proceeded in the absence of catalyst with water as the solvent. Experimental work and quantum chemical calculations were employed to better understand the role of the solvent and other factors affecting ring-opening and decarboxylation of pyrones. Our work in the upgrading of pyrones revealed that the presence of small amounts of biogenic compounds in the reaction mixture resulted in decreased catalytic activity over metal catalyst for the hydrogenation of pyrones. This inhibitive effect of biogenic compounds was systematically examined through the use of controlled additions of amino acids to the reaction mixture and observation of their effect on catalytic activity.

Achievements

We have previously reported that a rhodium-rhenium catalyst supported on carbon has been highly effective in the C-O hydrogenolysis of tetrahydropyran-2-methanol and tetrahydrofurfuryl alcohol to their respective α,ω-diols. To better understand the nature of the active site on this catalyst, we have obtained experimental reactivity profiles for a range of cyclic ethers and polyols. Examination of theses reactivity profiles suggested that the catalyst was bifunctional, possessing both metal and acid sites where C-O hydrogenolysis occurs first through acid-catalyzed dehydration or ring-opening, followed by metal-catalyzed hydrogenation to form the final product. Based on a Born-Haber cycle analysis, the relative reactivities of these molecules could be estimated using DFT-calculated gas-phase carbenium and oxocarbenium ion formation energies and compared against experimental trends. Figure 1 shows that there is good agreement between experimental data and theoretical calculations, and that the molecules we have examined may be classified according to three broad reactivity classes. The least reactive are those that form unstable primary carbenium ions, followed by those of intermediate reactivity which may form secondary carbenium ions or possess a neighboring hydroxyl group that results in the formation of primary oxocarbenium ions, and finally, the most reactive are those that form the most stable structures, i.e., secondary oxocarbenium ions.

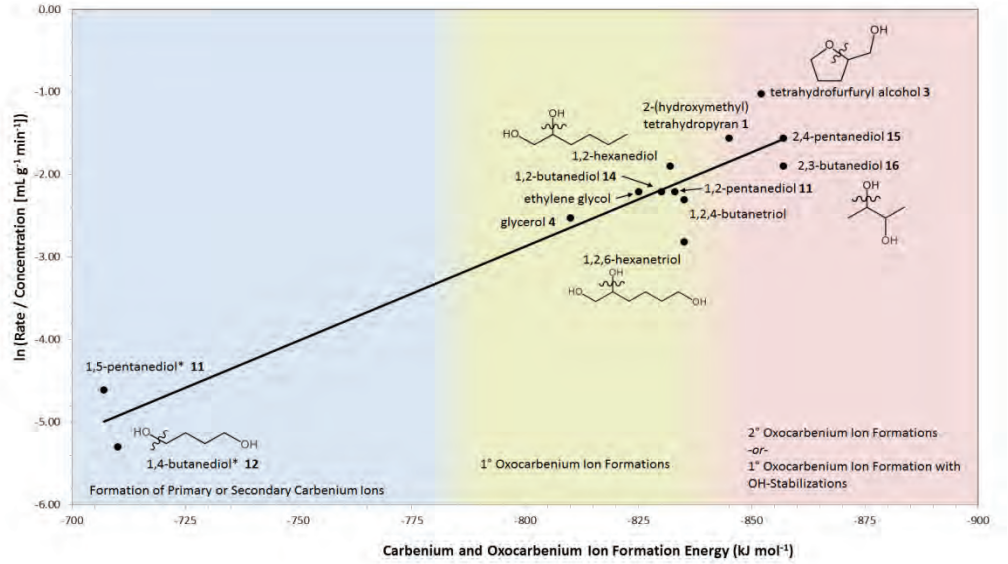


Figure 1. Comparison of the ratio between specific reaction rate (μmol g^{-1} min^{-1}) and initial reactant concentration (μmol mL^{-1}) (on a logarithmic scale) and DFT-calculated carbenium and oxocarbenium ion energies for selected cyclic ethers and linear polyols. Each region shows distinct shifts in activity due to the stability of the carbenium and oxocarbenium ions.

We believe that the unique Brønsted acidity for this catalyst originates from hydroxylated rhenium in close contact with metallic rhodium. The strong acidity of Rh-Re nanoparticles arises from strong Re-O bonds that form at the edges and corners of Re-Rh clusters, resulting in a weak O-H bond as well as high electron affinity for the conjugate base. Experimental evidence for acidity over this rhodium-rhenium catalyst has been obtained through two different ways. Ammonia temperature-programmed desorption was first used to determine the presence and density of acid sites as shown in Figure 2. Based on this technique, it was estimated that the catalyst has a total acid site density of 40 $\mu molg^{-1}$. A probe reaction was then selected to demonstrate that the catalyst possesses Brønsted acidity. For this purpose, the acid-catalyzed dehydration of fructose to 5-hydroxymethylfurfural was explored, and was found to occur over this catalyst in a continuous flow reaction system with activity and selectivity levels very similar to that of other solid acid catalysts such as zeolites, phosphated niobia, niobic acid, and zirconium phosphate (Figure 3). In summary, experimental reactivity trends, results from DFT calculations, catalyst characterization, and results from an acid-catalyzed probe reaction collectively support the hypothesis that selective hydrogenolysis of C O bonds in cyclic ethers and polyols over Rh-ReO_x/C takes place by acid catalysis, initiated by hydroxyl groups associated with rhenium.

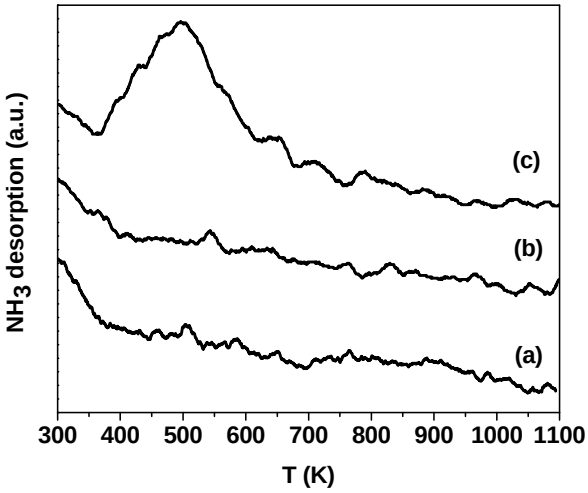


Figure 2. NH_3 temperature-programmed desorption profiles for (a) 3.6 wt% Re/C, (b) 4 wt% Rh/C, and (c) 4 wt% Rh-Re/C (1:0.5). Catalyst samples were pretreated in flowing H_2 (100 cm^3 (STP) min^{-1}) at 523 K for 4 h prior to dosing of NH_3.

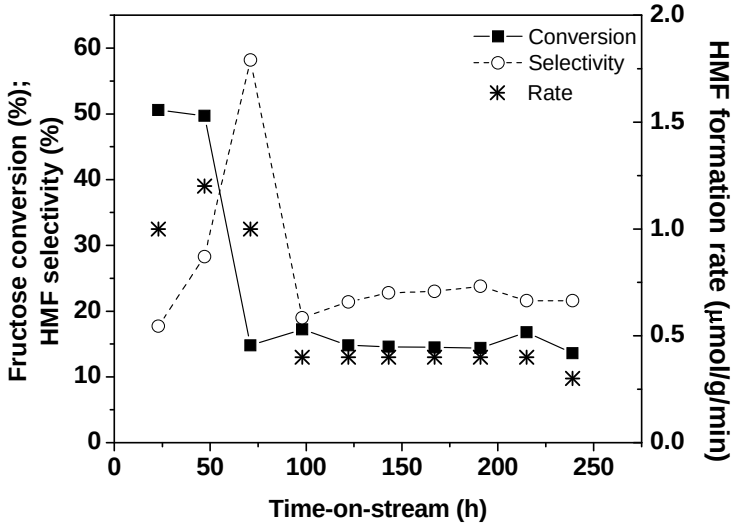


Figure 3. Dehydration of fructose to 5-hydroxymethylfurfural over 4wt% $RhReO_x/C$ (1:0.5). Catalyst samples were pretreated in flowing H_2 (100 cm^3 (STP) min^{-1}) at 523 K for 4 h prior to initiation of liquid feed (2wt% fructose in THF/H_2O (4:1)). Reaction conditions were 493 K, 300psi He, WHSV = 0.1 h^{-1}.

As part of efforts in the development of the Pyrone Testbed, we have previously performed exploratory studies using triacetic acid lactone (TAL), also known as 4-hydroxy-6-methylpyrone, as a feedstock for the production of several commodity chemicals (Figure 4). The catalytic transformations studied include selective hydrogenation, decarboxylation, dehydration, and acid-catalyzed ring-opening. Of the many end-products shown in Figure 4, sorbic acid (SA, **8**) could be obtained from TAL at yields of up to 64%. Notably, SA is a valuable commodity chemical which is widely used as a food preservative. Demonstration that SA can be produced from the pyrone, TAL, therefore indicates that TAL is a promising biorenewable feedstock for the production of value-added chemicals.

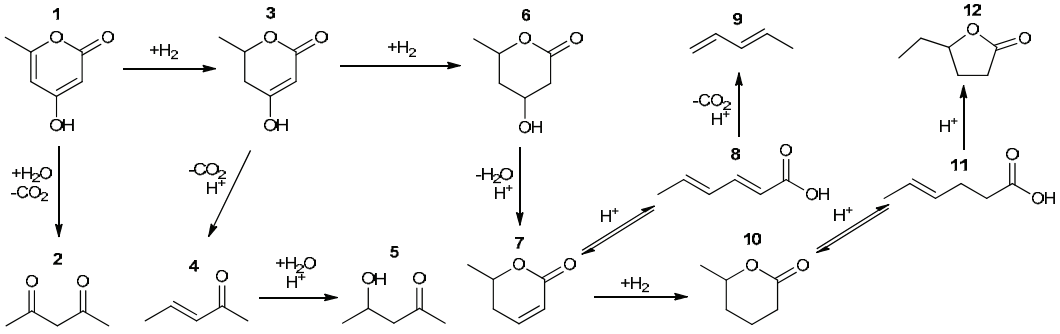


Figure 4. Reactions explored for the upgrading of pyrones to commodity chemicals. Compounds are as follows: 4-hydroxy-6-methyl-2-pyrone/ triacetic acid lactone (TAL, 1); 2,4-pentanedione/ acetylacetone (PDO, 2); 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one (DHHMP, 3); 3-penten-2-one (PO, 4); 4-hydroxy-2-pentanone (HP, 5); 4-hydroxy-6-methyltetrahydro-2-pyrone (4-HMTHP, 6); 6-methyl-5,6-dihydro-2-pyrone/ parasorbic acid (PSA, 7); 2,4-hexadienoic acid/ sorbic acid (SA, 8); 1,3-pentadiene (PD, 9); δ-hexalactone (HL, 10); hexenoic acid (HA, 11); γ-caprolactone (CL, 12).

Of the reactions depicted in Figure 4, the ring-opening and decarboxylation of TAL and DHHMP were selected for further study. These reactions were experimentally observed to proceed selectively in liquid water without the presence of a catalyst at low temperature (< 373 K), and have not been previously described in the literature. Addition of an acid catalyst in combination with water as the solvent resulted in increased reaction rates for DHHMP, while that for TAL were unchanged. The apparent activation energy barriers for the thermally-activated ring-opening and decarboxylation of TAL and DHHMP in water were measured to be 58 ± 12 kJ mol^{-1} and 42 ± 18 kJ mol^{-1} (95% confidence intervals), respectively. Preliminary results from quantum chemical calculations indicate that for both TAL and DHHMP, rearrangement of the molecule by keto-enol tautomerization to the keto-form of both pyrones likely occurs prior to ring-opening. For TAL, nucleophilic attack by water initiates ring-opening, followed by decarboxylation of the β-keto acid intermediate to form the dione product. In the case of DHHMP, after rearrangement of the ring to its keto form, it is proposed that concerted ring-opening and decarboxylation of the molecule occurs through a reto-Diels Alder mechanism, with CO_2 as the dienophile and the diene rearranging to form 3-penten-2-one as the final product.

Production of SA from TAL proceeds via hydrogenation of the unsaturated bonds in TAL and subsequent dehydration and ring opening of 4-HMTHP, as shown in Figure 4. Owing to the fact that TAL readily decarboxylates in water, as described above, we have taken the approach of using an adsorption scheme to recover TAL from spent fermentation media supernatant. The application of a simple polymeric adsorbent (Amberlite XAD-4, Dow) allows for adequate recovery of TAL; however, traces of amino acids are also recovered. We have observed that the presence of these compounds can result in a complete loss of hydrogenation activity. The use of an ion-exchange resin (Dowex Marathon C, Dow) removes the offending amino acids without altering the recovery of TAL and simultaneously allows for a recovery of catalyst activity.

The action of amino acids on hydrogenating metals is not well documented, so we have undertaken to study, qualitatively, the influence of these compounds by controlled addition to the reaction mixture. The degree to which TAL hydrogenation is inhibited is governed by the R-group of the amino acid. Six representative amino acids were investigated. Unsurprisingly, the sulfur-containing amino acids were the most inhibitory, while alanine was the least (containing only a methyl side chain). In order of decreasing effect when added at 0.05 mM, the amino acids studied are: Methionine (83% yield loss), Cysteine (77% yield loss), Tryptophan (71% yield loss), Phenylalanine (58% yield loss), Arginine (46% yield loss), and Alanine (25% yield loss). Even the addition of 0.01 mM of methionine results in a one third decrease in DHHMP yield. Consequently, there must be stringent guidelines on any separation process used for recovery of TAL (or any other biologically-synthesized compound) if a downstream catalytic hydrogenation is required.

Other Relevant Work

The work here for the rhodium-rhenium catalyst, and the efforts in selective dehydration by other projects in this Thrust, demonstrate the feasibility of obtaining commercially valuable α,ω-diols from biorenewable molecules. These results[1] provide the first consistent account for the nature of the active site in metal catalysts promoted with oxophilic additives for hydrogenolysis reactions, and provide guidance for the use of this new class of heterogeneous catalysts for the selective deoxygenation of biomass to fuels and chemicals. Work here for the pyrones demonstrates its

versatility as a platform molecule which supports the work performed by investigators in Thrust 1 and 2 which focuses on its production from biological routes. The ring-opening and decarboxylation of TAL and DHHMP has not been reported previously in the literature, and thus our efforts in this area will provide new insights for pyrone chemistry. The inhibitive effect of biogenic compounds on metal catalysts is vital in the Centre's focus on upgrading biologically-derived compounds, and provides guidelines for the downstream purification of these compounds prior to catalytic upgrading. To date, there is a scarcity of literature in this area; therefore the publication of our results will provide guidelines for the requirements of separation processes. A fundamental understanding of catalyst deactivation by biogenic impurities would allow for rational catalyst design which obviates the loss of activity that we have observed.

Plans for the Next Year

Detailed characterization of the Rh-ReO_x/C catalyst will be performed, with emphasis on the use of operando EXAFS to determine the oxidation state of Re, the coordination of Re with Rh and O, and how these catalyst properties vary under reaction conditions. Further computational and experimental work will be performed for the ring-opening and decarboxylation of pyrones to fine-tune mechanistic understanding in this area. While we have determined qualitatively the impact of amino acids on catalysts, a more quantitative study will be performed. Additionally, experimental work will focus on the nature of catalytic sites most susceptible to deactivation. Once an understanding of the site requirements is obtained, *in situ* FTIR will be used in conjunction with *operando* EXAFS and XANES to elucidate a molecular-scale picture of catalyst deactivation.

Expected Milestones and Deliverables

Characterization studies of the rhodium-rhenium catalyst using operando EXAFS will be performed in the next quarter. Experimental and computational work for the ring-opening and decarboxylation of pyrones will be performed in the next quarter. Quantitative studies of catalyst deactivation will be performed in the next quarter. A more detailed investigation of the site requirements for deactivation, including the types of reactions which are susceptible to deactivation, will be performed during the next year. Spectroscopic studies will follow these experiments.

Member Company Benefits

Members will have access to unpublished results from experimental studies.

Commercialization / Technology Transfer

TAL upgrading strategies have been disclosed to the Wisconsin Alumni Research Foundation (WARF), which is the technology transfer arm of the University of Wisconsin – Madison.

References

1. Chia, M.; Pagan-Torres, Y. J.; Hibbitts, D.; Tan, Q.; Pham, H. N.; Datye, A. K.; Neurock, M.; Davis, R. J.; Dumesic, J. A., Selective Hydrogenolysis of Polyols and Cyclic Ethers over Bifunctional Surface Sites on Rhodium-Rhenium Catalysts. *J. Am. Chem. Soc.* **2011,** 133, (32), 12675-12689.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.7** – Hydrothermally Stable Catalysts and Catalyst Supports

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Abhaya K. Datye	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Abhaya K. Datye, University of New Mexico

Other Faculty: James A. Dumesic, University of Wisconsin-Madison; Brent H. Shanks and Klaus Schmidt-Rohr, Iowa State University

Undergraduate Student: Amanda E. Anderson, University of New Mexico

Graduate Students: Yomaira J. Pagan-Torres, University of Wisconsin-Madison; Jason Anderson and Robert L. Johnson, Iowa State University

Postdoc: Haifeng Xiong, University of New Mexico

Research Assistant Professor: Hien N. Pham, University of New Mexico

Statement of Project Goals

The objective of this project is to develop catalysts and catalyst supports with improved hydrothermal stability in aqueous-phase reactions for bio-renewable conversion processes.

Project's Role in Center's Strategic Plan

A central challenge for the production of bio-renewable chemicals and fuels is the development of catalysts and supports that are hydrothermally stable during aqueous-phase reactions. Conventional catalysts and supports designed for gas-phase reactions may not be suitable for such reactions, particularly aqueous-phase reactions at temperatures in excess of 473 K. Specifically, loss of surface area, aggregation of the support and sintering or leaching of the metal phase could be significant issues. Hence, part of the catalyst tool chest for bio-renewable processing involves the development of stable catalysts and supports that can operate under aqueous conditions, with high activity and stability.

Fundamental Barriers and Methodologies

Mesoporous oxides, such as silica, are not hydrothermally stable at elevated temperatures due to grain growth and sintering resulting in loss of surface area. Likewise, solid acid catalysts, such as niobia, show loss of catalytic activity and stability in aqueous-phase reactions due to the transformation from amorphous to crystalline niobia, also resulting in loss of surface area. Previously, we have shown that by synthesizing high surface area mesoporous niobia by atomic layer deposition of niobia as thin films on the pore walls of mesoporous silica scaffold (SBA-15), we can significantly improve the hydrothermal stability of both niobia catalyst and SBA-15 support. In this project, we have explored another approach to improving the hydrothermal stability of the

SBA-15 support. This approach is based on coating the pore walls of SBA-15 with thin films of carbon by a more conventional technique, such as wet impregnation. We have also synthesized and tested acidic carbon catalysts for hydrothermal stability, and advanced solid-state ^{13}C NMR was used to characterize the carbon-based catalyst materials and their precursors in order to provide a more detailed structural explanation for their hydrothermal stability, which cannot be provided using standard characterization methods. Our approaches provide significant advance in the synthesis of hydrothermally stable catalysts and supports.

Achievements

Mesoporous SBA-15 Surface Coated with Carbon

An aqueous solution of sucrose was added to as-synthesized SBA-15, and the mixture was stirred at room temperature until water was evaporated. The dried powder was incompletely carbonized at 623 K for 2 h under UHP N_2 flow. The resulting carbon-silica material (25 wt% carbon) was subjected to treatments in liquid water in an autoclave vessel heated to 473 K at autogeneous pressure (22 bar) and held at this temperature for 12 h. To image the location of carbon in SBA-15, we acquired elemental carbon maps via energy-filtered transmission electron microscopy (EFTEM; Figure 1), where bright regions indicate the location of carbon in the EFTEM image at the Carbon K edge. The SBA-15 particle is shown as a dark region in the mapping, indicating that carbon is not present in uncoated SBA-15. The bright region in (b) is the holey carbon grid, which was used to prepare the samples for electron microscopy.

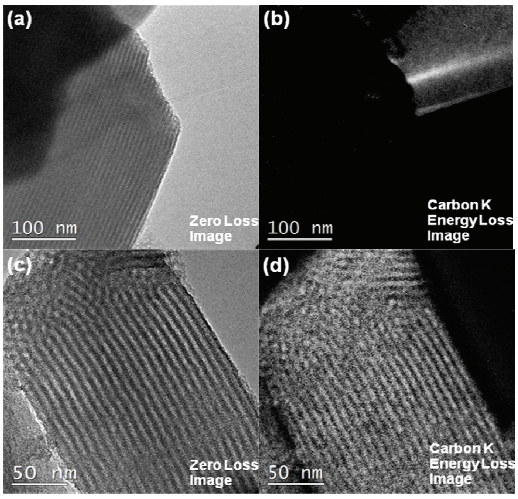


Figure 1. Zero loss images of (a) SBA-15 and (c) 25 wt% carbon/SBA-15; Carbon K energy loss images of (b) SBA-15 and (d) 25 wt% carbon/SBA-15.

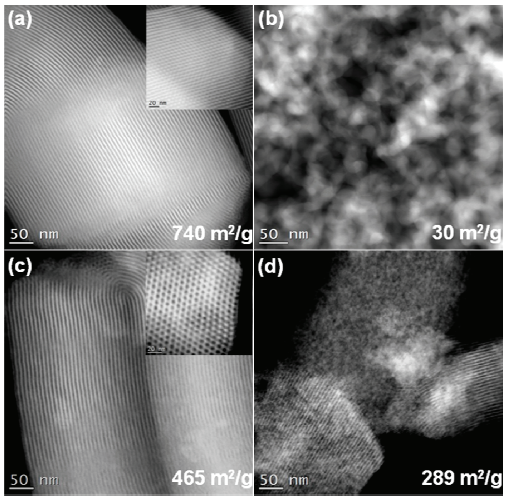


Figure 2. STEM images of as-synthesized (a) SBA-15 and (c) 25 wt% carbon/SBA-15; (b) SBA-15 and (d) 25 wt% carbon/SBA-15 after treatment in liquid water at 473 K for 12 h.

The zero loss image of carbon/SBA-15 (c) is similar to that of uncoated SBA-15 (a) in which we cannot directly observe carbon in SBA-15, whereas EFTEM image (d) shows bright lines corresponding to carbon on the silica pore walls and dark lines corresponding to the pores. This bright- and dark- line contrast confirms that carbon is located on the pore walls of SBA-15.

Scanning transmission electron microscopy (STEM) images of the as-synthesized SBA-15-based materials (Figure 2) show the retained hexagonal arrangement of pores after coating the pore walls

of SBA-15 with carbon. After treatment in liquid water at 473 K for 12 h, uncoated SBA-15 loses 96% of its surface area due to a complete collapse of the ordered mesopores, and therefore, loss of its structural integrity. In contrast, hydrothermal stability is significantly improved after coating SBA-15 with 25 wt% carbon, with a surface area loss of 38% and a mostly retained ordered mesoporous structure.

Hydrothermal Testing of Electrophilically Sulfonated Carbons

We have synthesized and tested acidic carbon catalysts for hydrothermal stability. Glucose was used to make four different sulfonated carbon materials: dry pyrolysis (350° and 450°C for ~10 h in N_2), hydrothermal carbonization (200°C for ~19 h in liquid water), and direct sulfonation of glucose (150°C for 2 h) by mixing with fuming sulfuric acid. This was compared with hydrothermal material sulfonated with sulfanilic acid for a total of five different catalysts. The hydrothermal stability of the catalyst was tested by treatment with 160°C liquid water for 24 h. Although they gave a >66% reduction in activity, the hydrothermal carbonization and directly sulfonated carbon catalysts showed the best hydrothermal stability after three hydrothermal treatments. Since standard characterization methods were unsuccessful in providing a structural explanation of the hydrothermal stability, we used advanced solid-state NMR.

Solid-State NMR of Carbon Catalyst Materials

We have developed and applied advanced solid-state NMR methods to characterize carbon-based catalyst materials and their precursors. The materials were prepared from ^{13}C-enriched starting materials, which provides 90-fold signal enhancement and enables ^{13}C-^{13}C correlation experiments. ^{13}C NMR can provide the most comprehensive structural information on carbon materials, identifying ketones, quinones, carboxylates, esters, phenols, furans, fused aromatic rings, alkyl linkers, and alkyl sidegroups. In addition, it can characterize the size of fused aromatic clusters, and (in)homogeneity on the 2- to 100-nm scale. A survey of various sulfonated carbon materials and their precursors by NMR (Figure 3), showed great complexity of the aromatic rings in the materials produced at moderate temperatures.

Hydrothermal char was chosen for particularly detailed investigations, since it is a scaffold rich in functional groups that are promising for attaching catalytic groups such as SO_3H. We developed new NMR techniques to selectively detect nonpolar arene groups and distinguish between furan and phenolic rings, which are virtually indistinguishable in standard unselective NMR. We also discovered a major diphenol (HO-C=C-OH) component, which explains the large phenolic-proton fraction deduced from titrations, and confirmed its structure using three-dimensional double-quantum ^{1}H-^{1}H-^{13}C NMR with suppression of C-H signals.

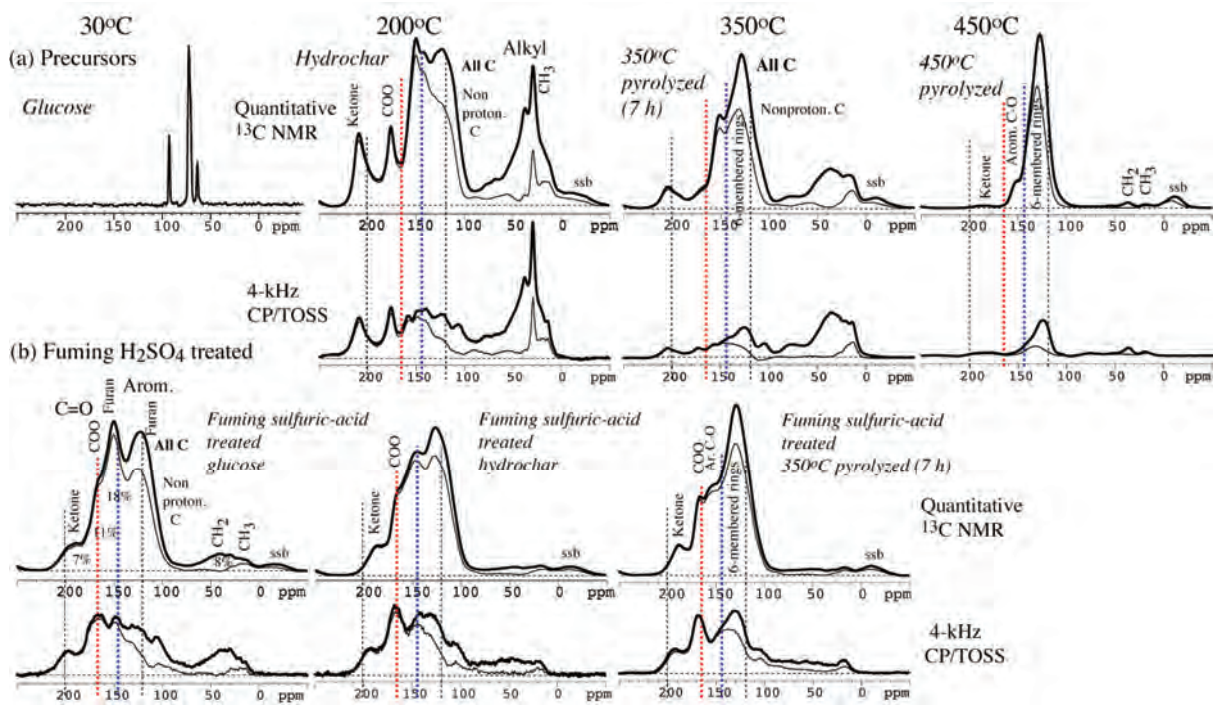


Figure 3. ^{13}C solid-state NMR of (a) unsulfonated precursors and (b) sulfonated carbon materials. Top row for each material: Quantitative spectra of all C (thick lines) and of nonprotonated or mobile C (thin lines). Bottom row: Spectral editing by slow (4-kHz) CP/TOSS, which suppresses most aromatic-carbon signals. Red dashed lines at 165 ppm mark unusual COO signals seen only in sulfonated carbon materials. Blue dashed lines at 143 ppm: Expected resonance position of aromatic C bonded to SO_3H groups.

Our studies further showed that aromatic C-H groups, which are the primary reaction centers for aromatic sulfonation reactions, are relatively rare in hydrothermal char and the other precursors studied. In turn, sulfonation was found to generate unusual COO groups in most materials studied; their exact molecular linkage needs to be studied further. Recently, we also started NMR analysis of carbon layers synthesized in SBA-15 mesoporous silica. Surprisingly, two-dimensional ^{13}C NMR showed that a material prepared at 300°C was inhomogeneous on the >2-nm scale.

Other Relevant Work

Hydrothermal stability is important for the long term industrial application of all of the chemical catalysts being developed within the ERC. Hence, improvements in the stability of the support can help provide the framework for catalysts for biorenewable conversions. Surface modification of silica is being also attempted by other groups. A recent report by Wang et al. (Materials Research Bulletin 46 (2011) 2187-2190) shows some improvement to the stability of MCM-41 silica with the addition of SiC. The resulting silica did not retain its ordered pore structure and the tests were done with boiling water, but not at elevated temperatures and pressures as done in this work.

Plans for the Next Year

We will explore carbon-silica materials as supports to deposit metal nanoparticles for use as catalysts in aqueous-phase reactions. In this case, small, uniform Pd nanoparticles will be deposited on carbon-silica by the alcohol reduction approach. The materials will be tested in acetylene hydrogenation reaction to determine the difference in selectivity towards ethylene between silica-

supported Pd catalyst and carbon-silica-supported Pd catalyst. In parallel, we will study the catalytic properties of Pd on carbon-silica supports in aqueous-phase reactions. We have started developing novel solid acid catalysts in which niobia is deposited as nanoparticles (< 20 nm) on carbon materials. We will do initial studies on the carbon-supported niobia catalysts for gas-phase isopropanol dehydration reactions, followed by studies in a biphasic reaction involving the conversion of glucose to HMF. Using the arsenal of new NMR techniques described above, the structure of sulfonated hydrothermal char and related materials will be characterized. Structural changes in the sulfonated materials due to hydrothermal stability tests will also be assessed. The acidic protons in the materials will be studied by ^{1}H NMR with high-speed magic-angle spinning (40-60 kHz). Comparison of the NMR analysis and thermal stability results will be used to elucidate the key chemical features required in carbon supports to maximize the hydrothermal stability of sulfonic acid groups on the support. Further, the functional groups in carbon layers on SBA-15 meosporous silica will be characterized.

Expected Milestones and Deliverables

1) Development of solid-state NMR methods for characterizing carbon-based supports
2) Use the knowledge gained from these advanced NMR techniques to determine the chemistry for synthesis of solid acid catalysts having a high degree of stability under hydrothermal conditions.
3) Catalytic tests with carbon coated silica supports

Publications currently in preparation:
"Two-dimensional ^{13}C Exchange NMR with C-H Spectral Editing for Characterizing ^{13}C-Enriched Low-Temperature Carbon Materials", Robert L. Johnson, Jason Anderson, Brent Shanks, Klaus Schmidt-Rohr.

"Phenolic Arene Cores and Diphenols as Central Structural Units of Hydrothermal Carbon Identified by ^{1}H-^{13}C and Spectrally Edited ^{13}C-^{13}C NMR", Robert L. Johnson, Jason Anderson, Brent Shanks, Klaus Schmidt-Rohr.

"Hydrothermal Deactivation of Electrophilically Sulfonated Carbon Catalysts", Jason M Anderson, Robert Johnson, Klaus Schmidt-Rohr, Brent H Shanks.

"Improved Hydrothermal Stability of SBA-15 Surface Coated With Carbon," Hien N. Pham, Amanda E. Anderson, Abhaya K. Datye.

Member Company Benefits

Hydrothermally stable solid acid catalysts have many potential applications in the conversion of bio-renewable feedstocks such as dehydration, esterification, and ring-opening reactions.

Commercialization / Technology Transfer

We plan to test these materials under demanding reaction conditions so that their commercial potential can be fully explored.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.9** – Pyrone Conversions

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
George Kraus	**2/17/12**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: George Kraus, Iowa State University
Other Faculty: Jim Dumesic, University of Wisconsin-Madison
Graduate Students: Jonathan Beasley, Sean Riley, Gerry Pollock Iowa State University

Statement of Project Goals

This project will develop efficient pathways to convert pyrones into industrial chemicals bearing an aromatic ring such as terephthalic acid.

Project's Role in Center's Strategic Plan

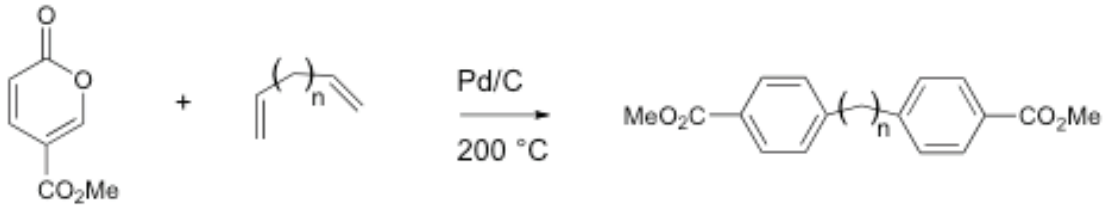
This project correlates the capabilities of Thrust 1 with the catalyst expertise in Thrust 3. It involves CBiRC company partners.

Fundamental Barriers and Methodologies

Presently, there are only a few reactions of pyrones that produce aromatic compounds. The key catalytic steps have been protected as disclosures and as provisional patents.

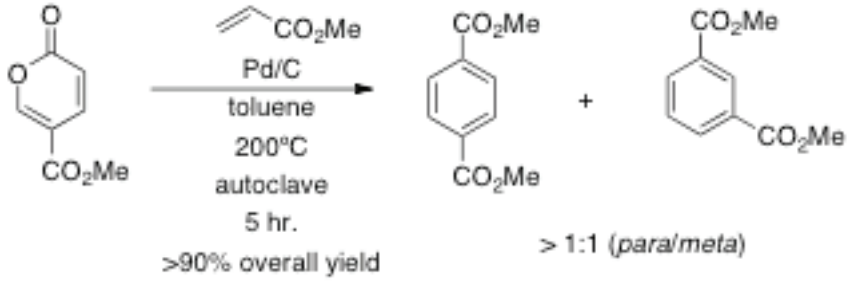
Achievements

To extend the cycloaddition protocol to bifunctional molecules, the Kraus group reacted the methyl ester of coumalic acid with dienes. In the initial case, the reaction of methyl coumalate with 1,5-hexadiene gave no product. However, the reactions of methyl coumalate with 1,7-octadiene or 1,9-decadiene afforded good yields of bifunctional compounds, as shown below. The Kraus group believes that oxidation of 1,5-hexadiene to an unstable triene appears to be faster than cycloaddition.



n = 4 66%
n = 6 72%
n = 2 0%

The Kraus group also investigated the reaction of methyl coumalate with acrylates. The case of methyl acrylate is shown below. The overall yield is high. Interestingly, the reaction produces a 1:1 ratio of the 1,3- and 1,4-disubstituted products. This is in contrast with the reaction with unactivated alkenes that provide only the para-substituted products. An effort to understand these results using computational chemistry has begun.



The Kraus group is currently studying steric and electronic parameters with the expectation that the ratio of 1,3- to 1,4- product can be shifted in favor of the para-substituted product.

Other Relevant Work

This project connects with the decarbonylation project to produce alpha-olefins which is part of Thrust 3. The cycloaddition chemistry of pyrones will extend the thrust's "tool chest" of catalytic methodology.

Plans for the Next Year

In the coming year, the Kraus group will continue to vary reaction parameters to improve the selectivity of the reaction of coumalates with esters and salts of acrylic acid. They will also study esters and salts of propiolic acid. This reaction has the advantage that the heterogeneous catalyst will not be needed.

The reaction of pyrones with hetero functional units will also be studied (for example methyl cyanoformate). This could allow the synthesis of pyridine containing compounds.

Expected Milestones and Deliverables

The Kraus group expects to provide an optimized pathway from malic acid to terephthalic acid that is amenable to scale up.

Member Company Benefits

Terephthalic acid is of interest to at least three CBiRC member companies. There are plans to submit proposals to federal funding agencies in collaboration with member companies to refine the process to terephthalic acid.

Commercialization / Technology Transfer

A provision patent for the process that produces alkyl benzoic acids from alpha olefins has been submitted. Disclosures for improvements in the conversion of malic acid to coumalic acid are expected in the coming year.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.10** – Selective Oxidation to Di-acids

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Robert Davis	**02/17/2011**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Robert Davis – Chemical Engineering, University of Virginia
Other Faculty: Matthew Neurock – Chemical Engineering and Chemistry, University of Virginia
Graduate Students:
Matthew Ide – PhD student, Chemical Engineering, University of Virginia
Sara Davis – PhD student, Chemical Engineering, University of Virginia
David Hibbitts – PhD student, Chemical Engineering, University of Virginia

Statement of Project Goals

The goal of this work is to understand the factors controlling the activity, selectivity and stability of heterogeneous catalysts for the selective oxidation of bi-functional molecules to produce di-acids. The project will use model polyol compounds for selective oxidation using O_2 as an oxidant at moderate temperatures in aqueous solvent.

Project's Role in Center's Strategic Plan

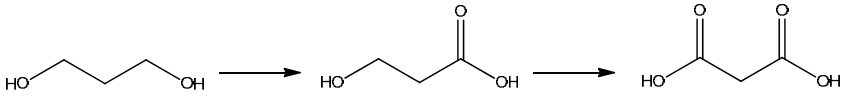
One of the integrative test beds in the ERC is the production of bi-functional molecules from sugars. The number of biologically-derived products possible with enzyme bioengineering and microbial metabolic engineering is numerous. These biologically-engineered chemical products formed by Thrusts 1 and 2 include ring compounds, alcohols, aldehydes, olefins and acids. Homogeneous and heterogeneous catalysts can convert these biological products to a wide range of useful chemicals through decarbonylation, hydrogenation, hydrogenolysis, oxidation, cycloaddition and dehydration. In this project, oxidation of alcohols and aldehydes is being pursued to produce di-acids, which are useful monomers in the plastics industry.

Fundamental Barriers and Methodologies

The selective liquid phase oxidation of polyols over heterogeneous catalysts is challenging because of the difficulty to oxidize the hydroxy mono-acid intermediate product. The first oxidation of a terminal alcohol group to an aldehyde and then to an acid group has been achieved by other groups, including our own. However, subsequent oxidation of another terminal alcohol group has proven difficult under conditions that are not highly basic.

The fundamental barrier of selective oxidation to di-acids in the absence of added base is unclear. The oxidation of glycerol (a model polyol) and hydroxymethylfurfural (derived from sugar) over supported Au catalysts in an aqueous solution effectively produces the hydroxy mono-acid at modest concentrations of base and di-acid at high concentrations of base. Unfortunately, supported Au

catalysts do not effectively oxidize polyols without the addition of base. When supported Pt and Pd catalysts are used for glycerol oxidation in the absence of base, the hydroxy mono-acid is produced but the catalysts suffer from rapid deactivation. Recent evidence in the literature suggests bimetallic supported catalysts containing Pt and the use of acidic or basic supports can produce some di-acid products. In addition, α,ω-diols have been successfully oxidized to hydroxy mono-acids using these catalysts in the absence of base. Therefore, the influence of diol chain length (3C, 4C, 5C, and 6C diols) and novel bimetallic supported catalysts containing Pt are being explored. The effects of oxygen pressure, pH, and temperature are also being investigated to explore why di-acids are not easily obtainable over traditional supported catalysts. A model three carbon α,ω-diol (1,3-propanediol) oxidation to hydroxy mono-acid (3-hydroxypropionic acid) and subsequent oxidation to di-acid (malonic acid) is shown below.



Achievements

The Davis group has investigated the oxidation of model α,ω-diol compounds over a commercial Pt/C catalyst. Table 1 shows the results for 1,6-hexanediol oxidation both with and without added organic acid. The organic acid was added to maintain a relatively constant pH during the course of the reaction.

Table 1. Oxidation of 1,6-hexanediol, with and without an organic acid additive.

Additive	pH	TOF s^{-1}	Conv. %
No Added Acid	6.5 to 3.5	0.27	29
No Added Acid[a]	6.5 to 3.5	0.28	15
0.35 M Acetic Acid	2.53	0.19	20
0.35 M Propionic Acid	2.55	0.18	20

Reaction Conditions: 0.1 M 1,6-hexanediol, 500 Sub.:Met. (mol:mol), T = 343 K, pO_2 = 10 bar, pH = 2.5
[a] 1000 Sub.:Met. (mol:mol)

When no organic acid was used the TOF was 0.27 s^{-1}, but the pH decreased significantly during the reaction, from 6.5 to 3.5. The use of an organic acid additive provided a relatively constant pH and more closely mirrored an industrial process where a portion of the product might be recycled to achieve a constant pH. The TOF decreased by approximately 30% when acetic acid or propionic acid was used as the organic acid additive. Figure 1 shows the effect of acetic acid concentration on the TOF of 1,6-hexanediol oxidation. In addition, the pH is shown on the secondary y-axis. A significant decrease in TOF was observed with increasing acetic acid concentration up to 0.2 M. A value of 0.35 M acetic acid was chosen to set the pH at about 2.5. It is unclear whether or not the decrease in rate of oxidation was the result of a decrease in pH or inhibition by organic acid adsorption on the catalyst surface.

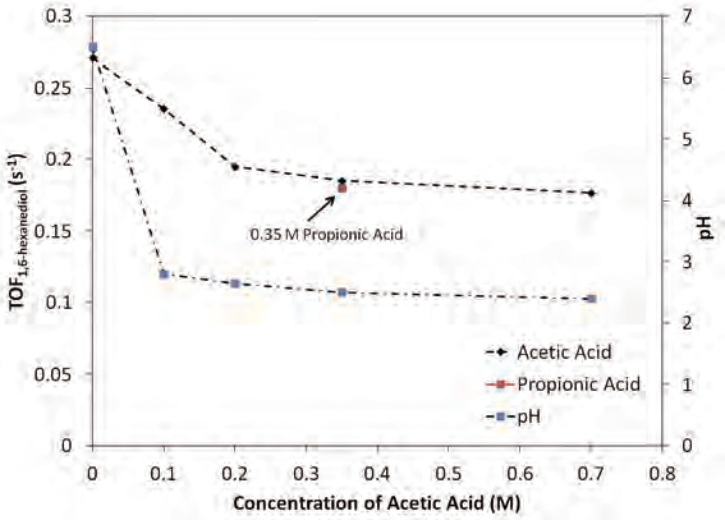


Figure 1. Effect of acetic acid on the TOF of 1,6-hexanediol oxidation. Reaction Conditions: 0.1 M 1,6-hexanediol, 500 Sub.:Met. (mol:mol), T = 343 K, pO_2 = 10 bar.

The oxidation of 1,3-propanediol (PDO) and 1,6-hexanediol (HDO) were performed at identical conditions and constant pH to compare their activity and selectivity to the di-acid product. Table 2 shows the oxidation rate and selectivity data. The experiments performed at a sub:metal ratio of 100 showed that at high conversion PDO was unable to selectively convert to the di-acid. The reaction was 89% selective to the hydroxy mono-acid (3-hydroxypropionic acid). However, 1,6-hexanediol was very selective to the di-acid at high conversions with 100% selectivity. The carbon balance was 99% for the PDO reaction and 85% for HDO. Interestingly, the only difference between the two substrates is the number of carbons between functional groups. The proximity of the hydroxyl groups apparently has an effect on di-acid selectivity.

Table 2. Oxidation rate and selectivity data for 1,3-propanediol (PDO) and 1,6-hexanediol (HDO) over Pt/C catalyst.

Sub:Metal mol:mol	Time min	TOF s^{-1}	Conversion %	Selectivity (%)			
				Aldehyde	Hydroxy Mono-acid	Aldehyde-Acid	Di-acid
PDO:Pt	15	-	14	52	48	-	0
100	1440		82	8	89	-	3
HDO:Pt	15	-	61	58	26	11	4
100	1440		100	0	0	0	100
PDO:Pt	15	0.05	4	80	20	-	0
500	240		11	54	46	-	0
HDO:Pt	15	0.19	19	92	6	2	0
500	240		45	65	25	8	2

Reaction Conditions: 0.1 M Substrate (PDO or HDO), T= 343 K, pO_2 = 10 bar, pH = 2.5

The rate of reaction was also significantly different for the two substrates. At a sub:metal ratio of 500 it was found that HDO had a TOF approximately four times higher than PDO. The role of hydroxyl group proximity within a molecule will continue to be explored by the Davis group with 4C and 5C diol substrates. In addition, a rapid deactivation of the Pt/C catalyst can be seen with both substrates. The conversion after 15 minutes was 4% for PDO and 19% for HDO, but only 11% and 45%, respectively, after 240 minutes. Deactivation is a major barrier for commercialization and thus was investigated in more detail.

The Pt/C catalyst used in all reactions was reduced for 4 hr at 473 K in 100 ml min^{-1} of H_2. The catalyst was found to have a 2.69 wt% of Pt by elemental analysis and a dispersion of 57% by chemisorption with H_2. As stated above, the Pt/C catalyst rapidly deactivated during the reaction after the initial fifteen minutes. One possible reason for deactivation is the leaching of Pt metal into the highly acidic solution. Table 3 shows the elemental analysis of reaction medium used for 1,6-hexanediol oxidation (low substrate to platinum metal ratio). An aqueous solution of 0.1 M 1,6-hexanediol was reacted with 0.065 g of 2.69 wt.% Pt/C at 343 K and 10 bar O_2. The initial pH of the sample was 6.5 and the final pH after 24 h of reaction time was 2.5 with a 69.3% yield of adipic acid (carbon balance of 69%). The final sample was filtered using a 0.2 µm filter and elemental analysis was performed by Galbraith Laboratories (Knoxville, TN) on the filtered liquid sample to determine the extent of platinum leaching.

Table 3 shows a concentration of 2.6 ppm of Pt was found in the liquid sample. Initially, an aqueous solution of 0.065 g of Pt/C with a 0.0269 weight fraction of Pt in 10 g of DI H_2O would have a concentration of 175 ppm if all Pt was leached. Thus, the overall percent of Pt that leached from the Pt/C catalyst during 24 h of reaction time was 1.5 %. This suggests that Pt leaching is not a significant contributor to the rapid deactivation of the Pt/C catalyst. In addition, the ratio of moles of adipic acid to moles of leached Pt was 3900. Thus, even a high concentration of strong carboxylic acid groups of adipic acid (pKa_1 = 4.43) did not cause significant leaching of Pt.

Table 3. Elemental analysis of used solution.

Sample Description	Wt.% of Pt in Solution
69.3 % Yield of Adipic Acid at 100% conversion at t = 1440 min.	0.00026
Maximum Pt Leachable into the Aqueous Solution	0.0195

Reaction Conditions: 0.1 M 1,6-hexanediol, 100 Sub.:Met. (mol:mol), T = 343 K, pO_2 = 10 bar

A second possible reason for Pt/C deactivation is the sintering of the Pt particles during reaction. A second reaction was therefore run with 0.1 M 1,6-hexanediol at 343 K and 10 bar O_2 and the platinum particle size distribution of the fresh and used catalyst was determined by HAADF STEM with a Titan TEM. The images were evaluated using ImageJ software. Table 4 shows that the particle diameter changed negligibly over the course of a 24 h reaction. A sample image is shown in Figure 2.

Table 4. Characterization of catalyst by TEM

TEM Particle Diameter Before Reaction	1.72 nm ± 1.20 nm
TEM Particle Diameter After Reaction	1.89 nm ± 1.45 nm

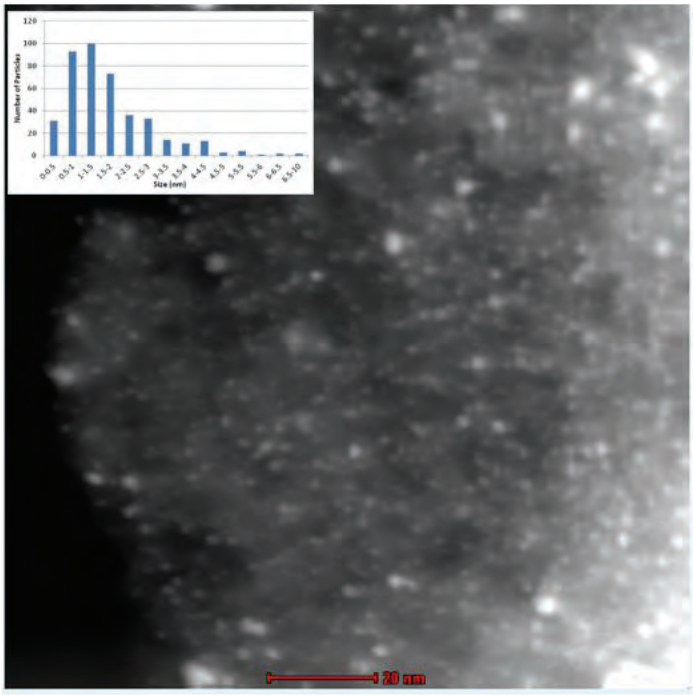


Figure 2. TEM image of fresh Pt/C catalyst. Pt particle diameter determined by ImageJ software.

Other Relevant Work

The field of α,ω-diol oxidation by heterogeneous catalysis with O_2 as the oxidant without a sacrificial homogeneous base has not been extensively explored. The literature has in it a few attempts at the oxidation of α,ω-diols with base in an aqueous solution, in organic solvents, and in the absence of base in aqueous solutions. The latter is of both industrial and academic importance as the effective oxidation of diols to di-acids over heterogeneous catalysts without the addition of a sacrificial base would allow the development of a cleaner and more economical process.

The catalytic oxidation of α,ω-diols with base in an aqueous solution has been reported over supported Au, Pt, and Pd. The oxidation of 1,3-propanediol with 1% Au/C and 1% Au/TiO_2 was reported by Biella et al. with 100% and 95% selectivity, respectively, to the monocarboxylic acid (3-hydroxypropionic acid).[1] Supported gold catalysts appear to be very selective towards the monocarboxylic acid product as the use of a 1% Au/C catalyst for the oxidation of 1,2-ethanediol produced 90% selectivity to glycolic acid.[2] In addition to supported gold's high selectivity for 1,2-ethanediol oxidation, Prati et al. showed no catalytic activity when 1% Au/C was used for the oxidation of glycolic acid. However, 5% Pt/C had a 40% selectivity to oxalic acid at 18% conversion and 5% Pd/C had a 50% selectivity to oxalic acid at 40% conversion at the same conditions as 1% Au/C.[3]

Samples of 5% Pt/C and 5% Pd/C were less selective in 1,2-ethanediol oxidation with 50% and 57% selectivity to glycolic acid, respectively, but both produced a 6% selectivity to the dicarboxylic acid (oxalic acid).[2] While large quantities of C1 products (formic acid and sodium bicarbonate) were formed, presumably by C-C bond cleavage, Pt and Pd were more selective than Au at α,ω-diol oxidation to the dicarboxylic acid. Recycling tests were performed for 1,2-ethanediol oxidation in the presence of base by Bianchi et al. They found that the activity of 5% Pt/C did not change after four recycles, but the 5% Pd/C catalyst had deactivated by 50% after the fourth run because of Pd metal

leaching into solution.[4]

The oxidation of an α,ω-diol in an organic solvent can significantly change the reaction products and selectivity. A 3% Au/TiO_2 catalyst with 5 nm particles oxidized 1,4-butanediol to γ-butyrolactone with 99% conversion and 99% selectivity in a tributyl phosphate (TBP) solvent.[5] Oxidative esterification of 1,3-propanediol in a methanol solvent has been reported over 1% Au/CeO_2 with 78.8% selectivity to methyl 3-hydroxypropionate at 98.1% conversion in the absence of base.[6] A similarly high selectivity of 83% to methyl glycolate was reported at 63% conversion of 1,2-ethanediol over 4.6% Au/Al_2O_3 in a methanol solvent in the absence of base. A 1% Pd/Al_2O_3 catalyst only had a 54% selectivity to methyl glycolate at 50% conversion.[7] In a 10% $NaOCH_3$ solution relative to 1,3-propanediol, Taarning et al. found a 90% selectivity to methyl 3-hydroxypropionate at 84% conversion over 1% Au/TiO_2.[8]

Despite high selectivity toward the hydroxy mono-acid (or lactone), oxidative esterificaton of diols over supported gold catalysts has low activity both with and without base. The oxidation of 1,3-propanediol had a TOF of 0.013 s^{-1} over 1% Au/TiO_2 (3.5 nm) with base at 373 K and a TOF of 0.015 s^{-1} over 1% Au/CeO_2 (3 nm) without base at 398 K.[6, 8] The rate is an order of magnitude slower than the TOF of 0.14 s^{-1} for 1,3-propanediol oxidation over 1% Au/TiO_2 at 343 K with base.[1] In addition, the 1% Au/TiO_2 catalyst (5.4 nm) had a TOF of 0.006 s^{-1} for 1,4-butanediol oxidation without base at 393 K.[5] Thus, even at high temperatures, the rate of oxidative esterification over supported gold catalysts is low both with and without base.

Recent literature has demonstrated that some heterogeneous catalysts are effective for the oxidation of both diols and polyols in the absence of base. However the rate, selectivity, and stability of the catalysts in acidic aqueous environment are unclear because the pH often changes over the course of the reaction. For example, the oxidation of 1,2-ethanediol was run using a 1% 1:3 AuPt/MgO catalyst with 25% selectivity to oxalic acid (di-acid) at 40.6% conversion,[9] but Mg can sometimes leach into solution under these experimental conditions and most likely acts as a sacrificial base. Prati et al. has performed the oxidation of 1,3-propanediol with a 1% 6:4 AuPt/H-mordenite and found an 85% selectivity to the hydroxy-monoacid (3-hydroxypropionic acid) at 41% conversion.[10] There is no mention of any di-acid being produced. Thus, while the oxidation of α,ω-diols has been somewhat successful over supported Pt catalysts, the literature has offered no explanation as to why they are ineffective at selective oxidation to the dicarboxylic acid.

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gold catalysts: methyl glycolate formation from ethylene glycol and methanol." *Catal Tod.* **2006**, 117, 210-213.

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10) Villa, A.; Veith, G.M.; Prati, L. "Selective Oxidation of Glycerol under Acidic Conditions Using Gold Catalysts." *Angew. Chem. Int. Ed.* **2010**, 49, 4499-4502.

Plans for the Next Year

The selective oxidation of bi-functional molecules to di-acids is a new project. The project will establish proof of concept by successfully oxidizing model bifunctional compounds to di-acids with commercial and novel heterogeneous catalysts in the absence of base. In addition, the role of the catalyst and the substrate will be investigated to understand the fundamental principles that determine selective oxidation to the di-acid. The most effective catalyst will be tested for stability, selectivity, and activity using compounds produced by Thrusts 1 and 2.

Expected Milestones and Deliverables

The project will explore the oxidation of diols to di-acids in the absence of base and present the work at the CBiRC annual NSF reviews, at working meetings, at conferences, and in published papers. Catalyst compositions, reaction rates, and performance evaluations will be reported to the center.

Member Company Benefits

Members will have access to results from experimental studies of selective oxidation reactions by supported metal catalysts. The member companies will also have access to catalyst synthesis and characterization techniques for novel supported metal catalysts.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: **T3.11** –Migration of Functional Groups

Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
L. Keith Woo	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: L. Keith Woo

Graduate Student: Gina M. Roberts, Iowa State University

REU Undergraduate: Philip Pierce, Virginia Polytechnic Institute

Statement of Project Goals

Organometallic strategies will be applied to the development of a catalytic toolbox for efficient conversions of renewable materials into chemical replacements for petroleum-based feedstocks.

Project's Role in Center's Strategic Plan

A key objective in this undertaking is to develop efficient and robust catalysts for the conversion of biosynthetic, short chain unsaturated fatty acids into bifunctional commodity chemicals. Representative targets include organic diacids. These dicarboxylic acids are useful precursors for the pharmaceutical and food industries. For example, preparation of fragrances, polyamides, adhesives, lubricants, and polyesters are some of the key applications of diacids.

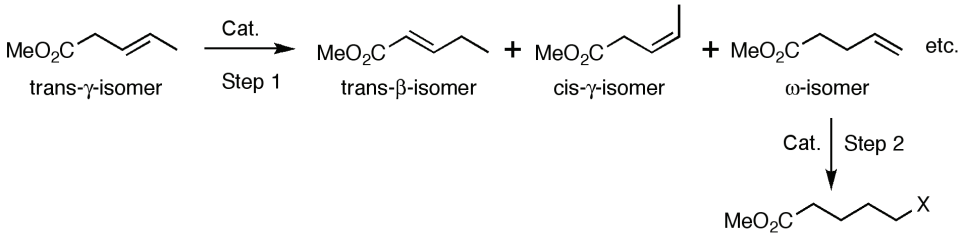
Fundamental Barriers and Methodologies

The efficient conversion of renewable natural resources, such as fatty acids, into useful industrial chemicals is an important means of reducing our dependence on crude oil. A goal of this project is to transform unsaturated fatty acids into value-added compounds. Potential targets are monomers for producing useful materials, such as polyesters, nylons, fragrances, and flavors. This would be a major step towards replacing petroleum products with biorenewable resources. For example, the industrial synthesis of adipic acid, a key monomer for producing nylon-6,6, is derived from benzene, a petroleum refinery commodity chemical. The project goal involves developing catalysts or catalyst systems that are capable of transforming monounsaturated fatty acid esters into derivatives with another functional group at the terminal (ω) carbon of the fatty acid. The double bond in the initial substrate may be anywhere along the carbon backbone. Moreover, if the biocatalysts in Thrust 2 produce a hydroxylated fatty acid, these compounds can be dehydrated to an unsaturated acid and esterified to provide the renewable feedstock for our chemical catalysts.

Current technology to convert unsaturated fatty acid esters involves a palladium catalyst that requires an expensive, air-sensitive phosphine ligand, high pressures of carbon monoxide, and high temperatures (130 ^{o}C). Our approach for addressing these issues is to replace the

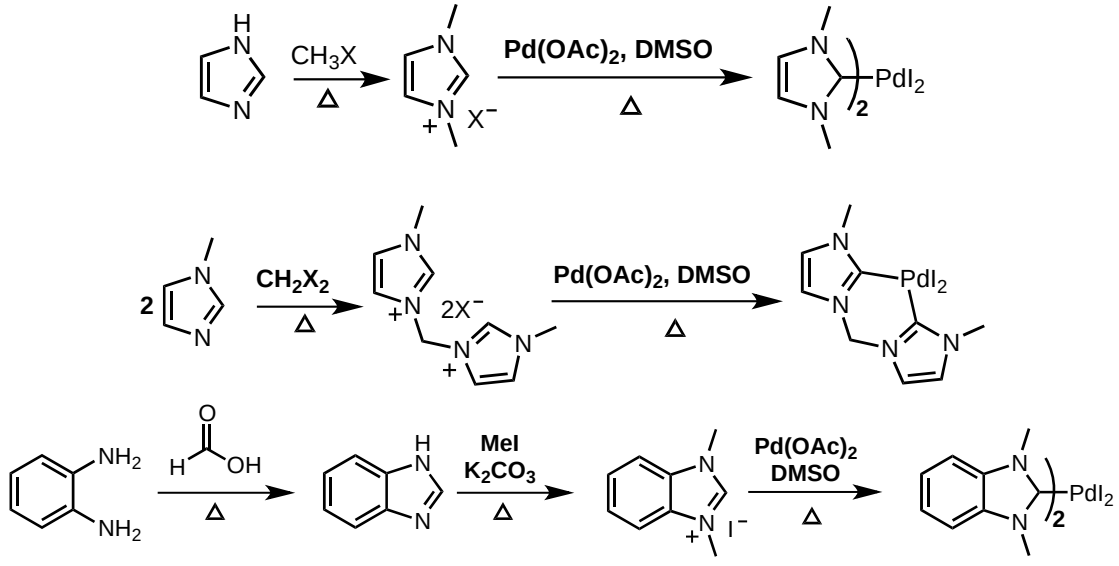
phosphine ligand with N-heterocyclic carbenes, NHCs. These NHCs have similar characteristics to phosphines, but are relatively easy to prepare, have tunable bulkiness and electronic properties, and are more robust that phosphines.

The catalysis approach that we are developing involves a one-pot, two-step process. In the first step, the double bond of the unsaturated fatty acid ester is rapidly isomerized along the hydrocarbon chain to produce a pool of all possible positional isomers. A second reaction only occurs when the double bond moves into the terminal position of the hydrocarbon chain. The selectivity of the second step results in a dynamic resolution of the mixture produced in step 1 such that only one double bond isomer is converted to product. The two reactions continue in tandem until the interconverting pool of double-bond isomers is transformed into one pure compound.



Achievements

Synthesis of the N-heterocyclic carbene (NHC) ligands was accomplished by alkylation of imidazole, methylimidazole or benzimidazole. The resulting imidazolium salts were readily



metallated by the addition of palladium acetate in the presence of base. The three Pd NHC complexes were evaluated for their activity as catalysts for the esterification of olefins (alkoxycarbonylation) in the presence of ethanol and carbon monoxide. Strong acid additives, such as methylsulfonic acid, led to the decomposition of the catalyst, presumably due to protonation of the NHC ligand and its removal as an imidazolium salt. However, weaker acids such as pyridinium methylsulfonate (PMS), successfully resulted in the conversion of olefins to esters. Of the three catalysts, the benzimidazole complex was extremely efficient at the esterification of 1-hexene, with yields as high as 93%.

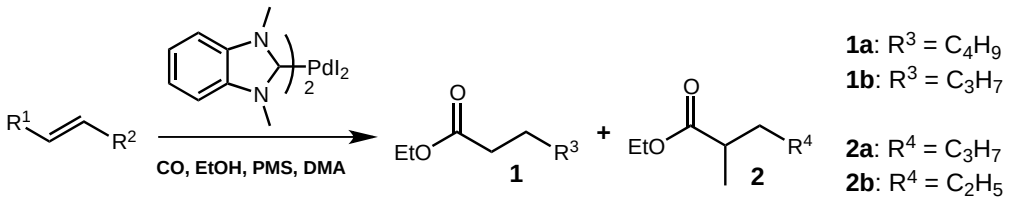


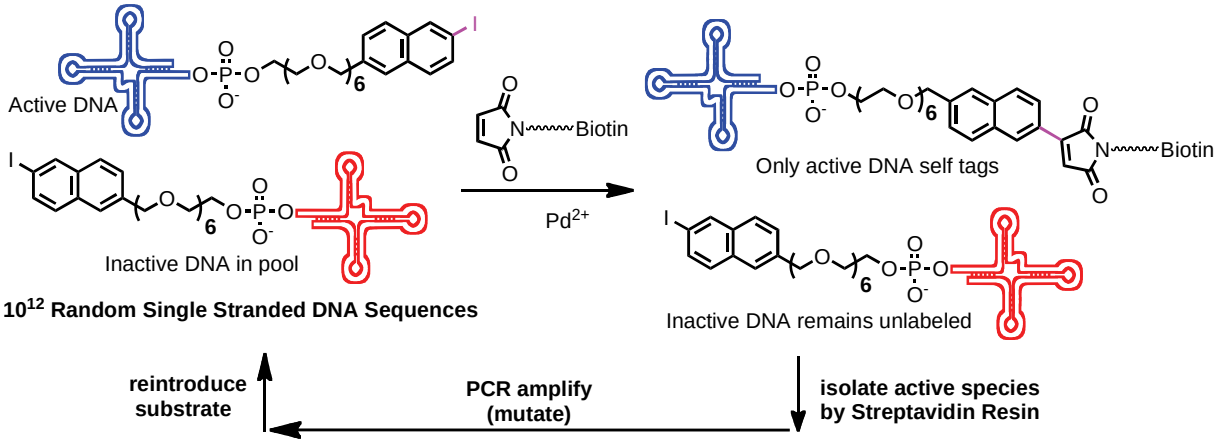
Table 1. Substrate and Solvent Variation[a]

olefin	% [Pd]	% PMS	t (h)	T (°C)	CO (psi)	Volume olefin	Volume EtOH	Volume DMA	Yield[b] (%)	Ratio **1**:**2**
1-hexene	1	15	24	110	1000	7.2	0.83	5.0	92.7	1:1.9
3-hexene[c]	1	15	24	110	1000	7.2	0.83	5.0	9.7	1:7.2
1-pentene	1	15	24	110	1000	6.3	0.83	5.0	25.2	0:1
2-pentene	1	15	24	110	1000	6.4	0.83	5.0	20.5	1:33

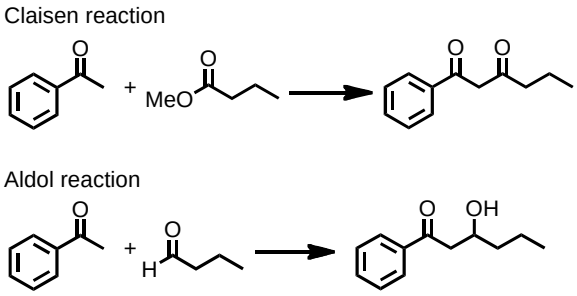
[a] Reaction prepared in an inert atmosphere, and carried out in a Parr reactor with the indicated time, temperature, and conditions. All reagents and solvents were dried and degassed before use. [b] Yields determined by GC. [c] The major product formed in this reaction is suspected to be ethyl 2-ethyl-pentanoate (~5.6%), not included in total yield.

Other Relevant Work

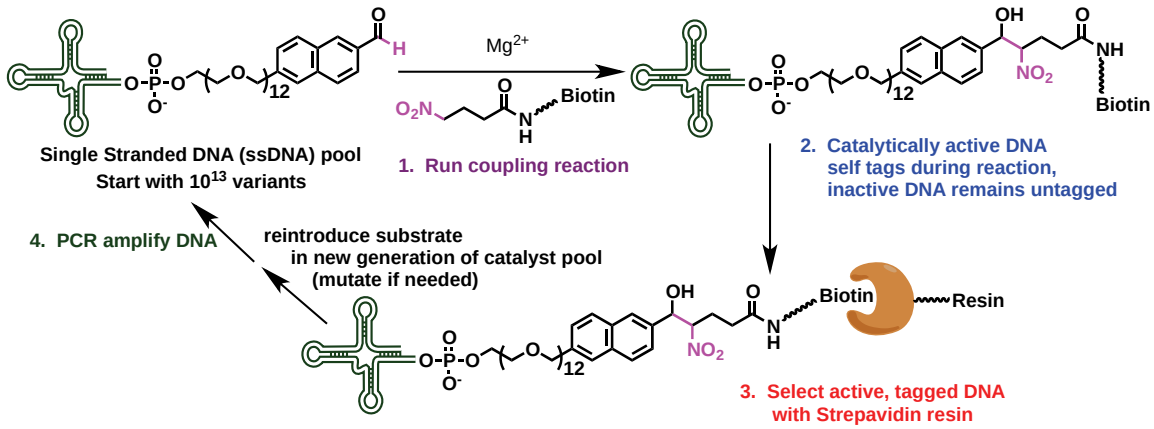
High-throughput strategies using iterative, evolutionary methods based on *in vitro* SELEX (Systematic Enhancement of Ligand by Exponential Enrichment) were applied to palladium catalyzed coupling reactions. Using single-stranded DNA 60-mers as active site scaffolds, we have demonstrated the feasibility of rapidly and efficiently optimizing a Pd^{2+}/DNA system for catalyzing the Heck coupling reaction between maleimide and iodonaphthalene. In eight rounds of SELEX, we were able to achieve 63% yields, representing a rate acceleration of greater than a 10^5-fold in coupling. This included decreasing the Pd^{2+} concentration from 1000 nM to 50 nM (95% reduction), lowering the process temperature to 25 °C, and shortening reaction times to 15 minutes.



We began work towards extending this high-throughput approach to the optimization of DNA-transition metal catalysts for the Claisen, aldol, and nitroaldol reactions. These reactions involve the coupling of two carbonyl compounds and are chemical analogs of the C-C bond-forming steps in the biological fatty acid synthesis cycle that is being re-engineered in Thrust 1.



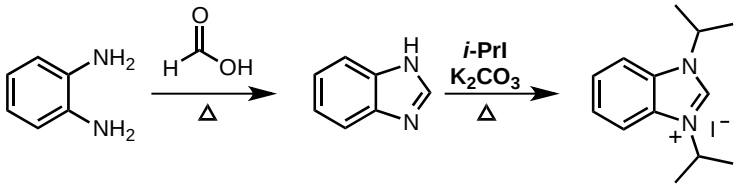
Several new bioconjugates were synthesized for the high-throughput development of the nitroaldol catalyst. The DNA-substrate linkage was synthesized using a phosphoramidite functional group.



Although the SELEX approach identified several DNA sequences that were active for the self-tagging nitroaldol reaction, application of these single-stranded oligomers in reactions with unattached substrates has not succeeded in producing true catalytic processes. Consequently, this work has been discontinued.

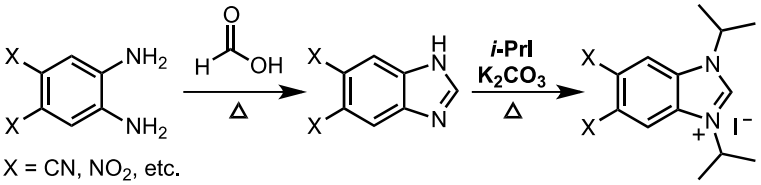
Plans for the Next Year

Work on optimizing catalysts for producing bifunctional chemicals will continue with the goal of addressing several issues. Although our Pd-NHC catalysts are capable of esterifying C-C double bonds in high yields, the regiochemistry currently favors the internal isomer over the desired terminal isomer. Control over the isomer selectivity should be possible by increasing the steric size of the NHC ligand so that the less bulky terminal product is preferred. Thus, isopropyl or larger nitrogen substituents will be introduced into the NHC ligand.



The isomerization efficiency for moving internal double bonds to the terminal position is also a necessary requirement of the desired catalyst system. An important criterion for promoting olefin isomerization is the generation of a metal hydride species. However, in our NHC-based complexes, this must be balanced against catalyst decomposition through reductive elimination of the imidazolidene ligand with the hydride to form imidazolium ions. Thus a study to determine the

appropriate acidity range for the catalysis will be undertaken. Complementary to this effort, we will also modify the NHC ligands with electron-withdrawing groups, such as cyano, nitro, etc., as a means to reduce its tendency towards reductive elimination.



We will also begin to examine other metal-NHC complexes in searching for more effective catalysts for the tandem isomerization-esterification process. Rhodium-NHC analogs will be examined next, as complexes of this metal are able to isomerize and carbonylate olefins.

Expected Milestones and Deliverables

New transition metal catalysts based on N-heterocyclic carbene ligands will be designed and optimized for the transformation of unsaturated fatty acid methyl esters (FAMES) into industrially useful bifunctional molecules. A key target will be the catalytic conversion of biologically derived short chain unsaturated FAMES into α,ω-diesters. These bifunctional molecules can serve as biorenewable replacements for petroleum-derived adipic acid in the industrial production of nylons.

Member Company Benefits

Member companies will have access to results on catalyst development for the conversion of unsaturated fatty acid methyl esters into α,ω-difunctionalized products.

Commercialization / Technology Transfer

If successful and applicable, the catalyst technology will be patented and offered for licensing.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: ERC – Small Business: Commercialization of Furanic-Based Biorenewable Chemicals (*a sponsored project*)

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adam Okerlund	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Adam Okerlund, Iowa State University

Other Faculty: George Kraus, Iowa State University

Undergraduate Student: Dalton Hughes, Iowa State University

Other Staff: Brendan Babcock, Iowa State University; Elliot Combs, Iowa State University

Statement of Project Goals

5-hydroxymethylfurfural (HMF) is a potentially valuable platform chemical that currently cannot be made cost-effectively. We have identified a cost-effective method for the selective dehydration of glucose and starch to produce HMF. The goal of this project is to **explore the techno-commercial potential** surrounding the processing conditions for HMF production within this new method. The cost-effective production of HMF will open the path to making furan-based building blocks used in production of plastics, fine chemicals, diesel fuel and fuel additives.

Project's Role in Center's Strategic Plan

CBiRC is striving to create an innovation ecosystem that supports the development of new technologies that could transform the petroleum-based chemical industry to a bio-based industry. Such emerging technologies often carry considerable techno-commercial risk and hence become opportunities for an early-stage company. This project was funded under the NSF 10-617 solicitation and the Grow Iowa Values Fund, creating an opportunity to found Glucan Biorenewables, a new startup entity. The project acts as a first example for CBiRC's innovation ecosystem through the formation of a translational research project supporting a new startup.

Fundamental Barriers and Methodologies

HMF is an intermediate step in the decomposition pathway of glucose to levulinic acid. Previous reports have shown enhanced glucose conversion and HMF selectivity in acidic aqueous systems combined with salts, particularly chloride salts such as MgCl2 and AlCl3, the application of pressure, and biphasic extraction. These promising results were the basis for the "ERC - Small Business: Commercialization of Furanic-Based Biorenewable Chemicals" grant.

While an ample amount of fundamental work has been performed on the dehydration of glucose to HMF, seldom has this work taken into account scale-up and ultimate production of HMF. New

work has been performed using water stable catalysts from the lanthanide series of rare earth elements in a biphasic reaction system. This biphasic reactor consists of an aqueous layer saturated with a salt, typically NaCl. The methodology of this project focuses on finding a using best-case (least-cost) reaction scenario for HMF production based on capital investments as well as consumable expenses.

Achievements

The first year of this project has focused on probing existing information to advance the understanding of the biphasic reactor system and how it could become amenable to industrial scale HMF production. After reviewing current information, the decision was made to focus on the water tolerant lanthanide series. These rare earth elements serve as Lewis acid catalysts and are viable without pH adjustment. The ability to perform the glucose dehydration reaction at a neutral pH eliminates the costly specialized equipment necessary at scale for highly acidic environments.

Other notable conditions for the previous lanthanide series work were the biphasic reaction system and the saturation of the aqueous phase with salt. Recent work has shown promising HMF extraction results by use of alkylphenols, such as sec-butyl phenol. While alkylphenols may extract HMF well for the aqueous phase, they are not ideal solvents for the reaction when thinking about the entire process. Both HMF and alkylphenols have high boiling points with HMF is a liable to degrade at high temperatures. It became evident that a lower boiling point solvent should be used as the organic extraction phase for potential removal via distillation. Several hydrophobic solvents were examined for use as the organic extraction phase. It was determined that tetrahydrofuran (THF) would be a good choice for the extraction solvent moving forward as it had high HMF extraction capabilities as well as a low boiling point. The majority of the reactions for this project to date have used THF as the extracting solvent.

The saturated salt conditions offered another avenue for experimentation. While the high salt content isn't ideal, it plays a crucial role in partitioning. The salting-in and salting-out effects of HMF by the salts mirror the Hofmeister series which is commonly used for protein solubility. Employing high salting-out salts such as Na_2SO_4 are great for partitioning, but can detriment the glucose to HMF conversion. Experimentation is still underway to determine what role and how much of a role the salts play in the catalytic conversion of glucose to HMF with the system we have designed.

Other Relevant Work

The production of HMF from biorenewable feedstocks has become a hot topic over the last decade. Some groups have focused on using fructose as a feed stock due to higher yields while others work with glucose and polysaccharides. The reaction systems for the saccharide conversion to HMF vary and include, but are not limited to, ionic liquids, supercritical water, biphasic liquid-liquid, and acidic aqueous conditions.

The James Dumesic (University of Wisconsin-Madison) and Brent Shanks (Iowa State University) groups within CBiRC are working on related glucose dehydration projects.

Plans for the Next Year

The plan for the next year is to:

1) Finalize and verify experiments on salt effects of the dehydration of glucose to HMF in our biphasic batch reactor system.
2) Pursue moving from a batch reactor system to a continuous process more suited for large scale production.

Expected Milestones and Deliverables

- March 2012 – August 2012: Finalize and verify experiments on salt and solvent effects of the dehydration of glucose to HMF in our batch reactor system
- August 2012 – March 2013: Develop and demonstrate glucose to HMF conversion capabilities in a continuous flow system

Member Company Benefits

This project is connected through its funding to Glucan Biorenewables, one of the member companies working closely with CBiRC. This translational research project allows the startup entity to move towards a further proof of concept for the technology.

Commercialization / Technology Transfer

Glucan Biorenewables has taken a license to the base technology at the core of this project. Additional know-how is expected as the project moves forward.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Furanics-Based Biorenewable Chemicals (*a sponsored project*)

Thrust: Research Thrust 3 – Chemical Catalyst Design

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adam Okerlund	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Adam Okerlund, Iowa State University

Undergraduate Student: Dalton Hughes, Iowa State University

Other Staff: Brendan Babcock, Iowa State University; Elliot Combs, Iowa State University

Industrial Participants: Glucan Biorenewables, Inc.

Statement of Project Goals

Research and development at CBiRC has identified a cost-effective method for the selective dehydration of glucose and starch to produce HMF. We must now implement a cost-effective and scalable downstream separation and purification strategy for HMF. The goal of this project is the research and development of the cost-effective **purification strategy** with the ultimate goal of obtaining high purity HMF.

Project's Role in Center's Strategic Plan

CBiRC is striving to create an innovation ecosystem that supports the development of new technologies that could transform the petroleum-based chemical industry to a bio-based industry. Such emerging technologies often carry considerable techno-commercial risk and hence become opportunities for an early-stage company. This project was funded under the NSF 10-617 solicitation and the Grow Iowa Values Fund, creating an opportunity to found Glucan Biorenewables, a new startup entity. The project acts as a first example for CBiRC's innovation ecosystem through the formation of a translational research project supporting a new startup.

Fundamental Barriers and Methodologies

HMF is an intermediate step in the decomposition pathway of glucose to levulinic acid. Previous reports have shown enhanced glucose conversion and HMF selectivity in acidic aqueous systems combined with salts, particularly chloride salts such as MgCl2 and AlCl3, the application of pressure, and biphasic extraction. These promising results were the basis for the "Grow Iowa" Values Fund - Furanics-Based Biorenewable Chemicals grant. While the biphasic reaction of saccharides to HMF has been broadly researched and developed, the purification of HMF from an organic extraction phase has not.

Information and know-how for the cost-effective HMF production will be obtained from other Thrust 3 projects within CBiRC. Most of the current production methods employ a biphasic system

where HMF preferentially resides in the organic phase. We must now extract the HMF from this organic phase in high yields and at low cost. The methodology of this project focuses on finding a best-case (least-cost) purification scheme for HMF from the organic extraction phase based on capital investments, consumable expenses, and energy costs.

Achievements

The first year of this project has focused on researching several purification strategies of HMF from the organic phase of the biphasic reactor system. Care was taken to only research strategies that were potentially feasible at an industrial production scale. After reviewing pertinent literature and examining the chemical properties of the HMF versus the organic extraction phase, humins, and other contaminants, the decision was made to focus on the three means of purification; liquid-liquid extraction, adsorption onto a solid phase, and distillation.

Distillation of HMF from an alkylphenol extraction phase was the first means of separation attempted in the laboratory. The high boiling points of HMF and alkylphenols, the presence of humins, and the reactivity of HMF at high temperatures combined to make this a very poor purification strategy. With HMF reactivity at high temperatures, the removal of a low boiling point organic extraction phase via distillation makes for a better option. Subsequent reactions now use tetrahydrofuran as the organic extraction phase. This is better, but humins and other high boiling point contaminants remain in the distillation bottoms along with HMF.

Purification was also attempted via adsorption onto solid phase resins. A variety of ion exchange, polyethyleneimine, and silica based resins were employed to bind either HMF or a portion of the contaminants. Minimal separations of HMF from its contaminants occurred with both the ion exchange and polyethyleneimine resins, but a scheme was devised for purification on the silica resins. This scheme must still be developed before it can become a practical process at scale.

The third means of purification attempted for HMF was by liquid-liquid extraction with water from the post reaction organic extraction phase. HMF has high solubility in pure water. The salt used during the glucose to HMF dehydration reaction helps to facilitate the separation of the phases and also helps to lower the solubility off HMF in the aqueous phase. After the reaction is completed, the aqueous phase saturated with salt is removed and replaced with fresh water. The ability to perform this type of purification is dependent on the polarity of the extraction phase. If the organic extraction phase is too polar, it becomes miscible with water in the absence of salt eliminating liquid-liquid extraction possibilities. Another liquid-liquid option which has shown success is the addition of both water and a nonpolar solvent such as hexane to the organic extraction phase. The hexane helps to lower the solubility of HMF in the organic extraction phase as well as facilitate a biphasic separation in the absence of salt.

Other Relevant Work

The production of HMF from biorenewable feedstocks has become heavily researched over the last decade, but little advancement in a purification strategy has been developed. Partial purification has been achieved by other groups by using adsorption onto acidic ion exchange or non-functional polymeric resins, binding to soluble polyethyleneimine, and distillation.

Plans for the Next Year

The plan for the next year is to:

1) Finalize a strategy for HMF production in a biphasic reactor via another CBiRC project.
2) Apply the knowledge gained from previous HMF separation and purification experiments to develop a final purification process with scale up potential.
3) Perform cost analysis of the entire HMF process.

Expected Milestones and Deliverables

- March 2012 – August 2012: Finalize a strategy for HMF production in a biphasic reactor via another CBiRC project.
- August 2012 – March 2013: Apply the knowledge gained from previous HMF separation and purification experiments to develop a final purification process with scale up potential.
- March 2013 – May 2013: Perform cost analysis of the entire HMF process.

Member Company Benefits

This project is connected through its funding to Glucan Biorenewables, one of the member companies working closely with CBiRC. This translational research project allows the startup entity to move towards a further proof of concept for the technology.

Commercialization / Technology Transfer

Glucan Biorenewables has taken a license to the base technology at the core of this project. Additional know-how is expected as the project moves forward.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Techno-Economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars

Thrust: Research Support – Life Cycle Assessment (LCA)

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Robert Anex	**03/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Robert Anex (University of Wisconsin), Sampath Gunukula (University of Wisconsin), Raj Raman (Iowa State University), Carol Faulhaber (Iowa State University), Amanda Culp (REU student, University of Pennsylvania), Monica Dahl (REU student, Manhattan College)

Statement of Project Goals

The objective of this study is to evaluate the techno-economic feasibility of hydrocarbons produced from low-cost biomass-derived sugars with particular emphasis on evaluating and informing the development of our testbeds. The testbed technologies are in early development so only preliminary experimental information is available that informs the likely operating conditions of the major unit processes. However, it is precisely at this early stage of development that we need to identify the major bottlenecks and research priorities that will make these processes economically feasible and direct our research efforts accordingly. We have developed methods for early-process 'bounding analyses' that allow us to screen technology pathways. We have three main activities in this project: 1) developing methods for early screening of process economic and technical feasibility; 2) developing detailed models and databases of processes early in the life-cycle that are common to all chemicals and will be needed for detailed analysis as technology pathways are more fully defined; 3) evaluation of alternative routes to our target chemical platforms.

Project's Role in Center's Strategic Plan

This project is central to achieving the Center's strategic objectives. The Life Cycle Assessment (LCA) support area includes development and application of a variety of assessment methods that guide the research and development direction of the individual thrusts and the Center's overall priorities. Analysis of the techno-economic feasibility and environmental impact of proposed technology pathways from biorenewable resources to chemicals will identify technology bottlenecks and environmental constraints that must be addressed through research or system reconfiguration. The LCA activities in evaluating the testbeds also provides vehicle to integrate the thrusts and individual projects of the center. Understanding the trade-offs that are inherent in choices made within the thrusts requires an undestanding of the full center mission and technology life cycle. Thus the LCA analyses are carried out in close collaboration with the engineers and scientists working in the thrusts and foments a deep and meaningful discussion among the thrusts leading to a truly transdisciplinary understanding of and approach to the Center mission.

Fundamental Barriers and Methodologies

One fundamental question for this Center is how to predict during the earliest stages of development of a chemical pathway its technical, economic and environmental feasibility. The Center has the capacity to develop thousands or perhaps tens of thousands of pathways, but we must screen these at the earliest stages of development and select only the most promising for development. One dimension of this screening requires techno-economic and life-cycle assessment methods that can be discriminate between pathways.

Another fundamental question for CBiRC is: at what point chemicals developed by the biological catalyst platform should be handed off to the chemical catalyst platform. A first step to understanding this is to develop models of the technical and economic dimensions of converting the intermediates via chemical catalyst to valuable end products (e.g., alkanes) so that different intermediate starting points can be evaluated.

Achievements

We have developed a method for screening and framing the economic feasibility of biorenewable chemicals at very early stages of development. We have applied this method to the two testbeds currently active in CBiRC. For example, under the carboxylic acid testbed we have examined the production of α-olefins. We analyze the biological production of carboxylic acids (i.e., short chain fatty acids) from glucose and subsequent catalytic conversion to α-olefins through hydrogenation to alcohols and subsequent dehydration. The production cost estimate is based on the overall yields of the two processes areas and the cost of glucose feedstock. Based on theoretical carbon yields at each stage we can determine the minimum production cost of product based on feedstock cost. We then adjust the process, adding more practical yields, and modeling specific processes such as separations to add operating and capital costs. Ultimately, we can predict a minimum production cost and compare that with product cost targets to assess economic feasibility. For example, we estimate a minimum production cost for α-olefins (modeled as C6-C8 chain length) of $0.47/lb based on an input glucose cost of $200/MT compared to an approximate market value of petroleum-derived α-olefins of $0.50/lb.

In order to be able to analyze a wide range of chemical pathways rapidly at early stages of development we have produced a series of generic unit process models. For each unit process (e.g., fermentation) we have developed a series of models that range from very simple to complex. The simplest models require little input data but yield a less certain output and more complex models require much more detailed input information, but yield more certain output predictions. We can use these models in an iterative manner in our techno-economic analysis of the testbeds, beginning with the simplest models for screening and moving to the more complex models as experimental work provides more detailed data on the likely performance of the unit processes in our testbeds.

In the 2011 reporting period we have built a fairly simple engineering-economic model that considers how the cost of fermentation is influenced by fundamental biokinetic parameters (e.g., maximum specific growth rate, yield), to enable conversations within Thrust 2 about how metabolic engineering tradeoffs impact overall product cost. We developed a series of rough functional relationships between key biological parameters and product cost, based on the thesis work of a CBiRC graduate student. These allowed us to initiate discussions with test-bed leaders on the biokinetic targets. For example, in the figure below, the importance of overall process yield on minimum selling price is evident:

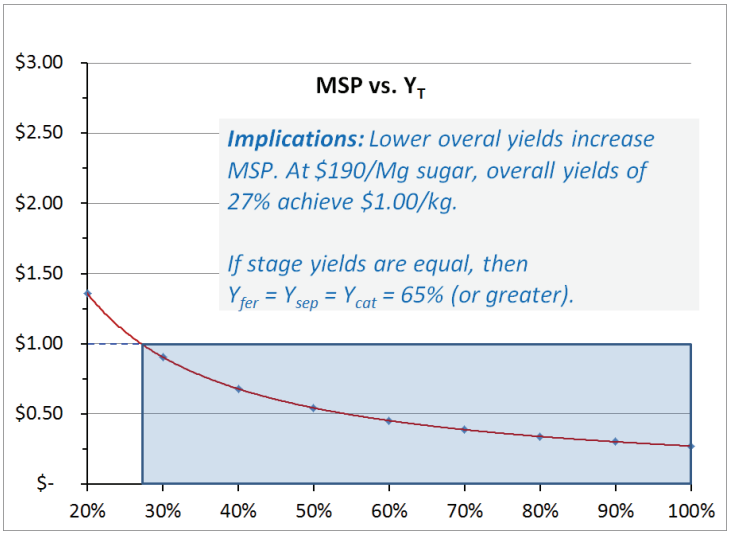


Figure 2. Minimum Selling Price (MSP) is strongly influenced by overall process yield. As overall yield goes down, MSP increases rapidly. Using a $1.00/kg target (and assuming that 70% of product price is feedstock, as is true for large-scale ethanol production), a minimum overall yield of 27% is required. This appears low until one realizes that overall yield is cumulative, and that the fermentative yield (Y_{fer}) for several CBiRC processes is less than 50%.

Similarly, fermentation productivity (g/L/h), is crucial to minimum selling price. Assuming a 1000 m^3 reactor, total capital computed and amortized over 15 years @ 15%, and ignoring operating and maintenance costs, the minimum selling price vs. productivity curve shown in figure 3 was generated.

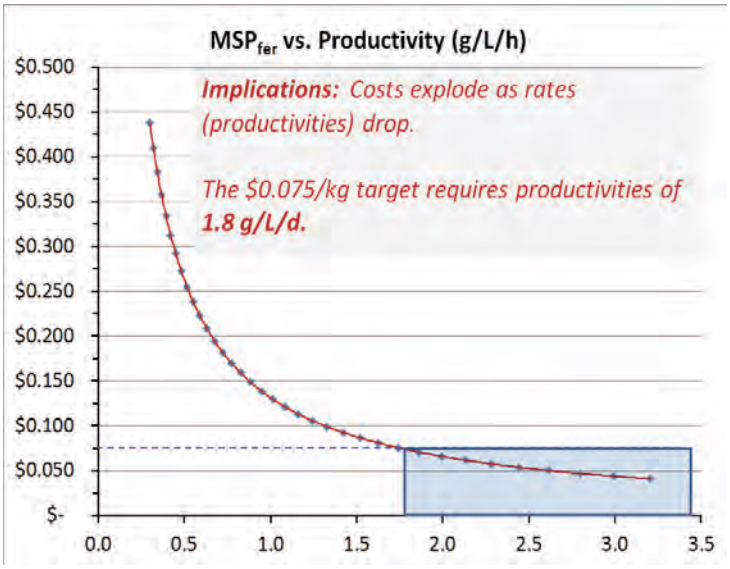


Figure 3. Minimum Selling Price (MSP) is strongly influenced by fermentation productivity. As productivity increases, MSP decreases rapidly. Using a $1.00/kg target MSP, assuming that 70% of product price is feedstock, with the remainder being processing cost, and assuming that 25% of the processing cost is for fermentation, with the remainder being separation and catalysis, a minimum productivity of 1.8 g/L/h is recommended.

We have also developed a set of detailed techno-economic models for a set of catalytic processes that are representative of the conversion of intermediate chemicals being developed in Thrust 2. The development of these models has answered important questions about the feasibility of scaling up such processes, and has also allowed us to develop and test our modeling and analysis techniques for the CBiRC testbed processes.

We also developed a method for screening CBiRC chemicals based on their chemical structure. This QSAR-based method was developed over the summer by two REU students and used to screen the intermediate and final chemicals in the pyrone testbed. The QSAR methods were also used to develop LCIA factors for the CBiRC chemicals. In combination these methods were presented at the LCA XI meeting in Chicago, IL and a poster was presented at the REU conference at the University of Wisconsin, Madison. Of the 113 CBiRC chemicals evaluated using the QSAR methods, only four were determined to be non-biodegradable but 17% were found to have high risk of ecotoxicity.

Other Relevant Work

Many organizations have made significant investments in biofuel and biorenewable chemical technologies and naturally many of these organizations are assessing the economic and technological feasibility of the technologies that they are studying. These efforts are mostly focused on specific pathways rather than taking a more general approach and assessing the potential of technology platforms and classes of biorenewable products. An example of the more narrow approach is a recent partnership between ConocoPhillips Company, the National Renewable Energy Laboratory and Iowa State University performing techno-economic analysis of the near-term (5-8 years) potential for liquid fuels from biomass via gasification, pyrolysis and biochemical conversion. The leader of the CBiRC LCA thrust, Dr. Robert Anex, participated in this study. The biofuel assessment provides complementary capability through detailed models of fuel conversion technologies likely to be incorporated in the first generation of biorefineries.

There is significant interest in biorenewable chemicals and this has spawned a number of LCAs of specific technologies. For example, a recent study examined the production of ethylene from sugar cane in Brazil. These studies are worthwhile but differ from the CBiRC efforts in LCA in that they address specific biorenewable products, rather than a platform of chemicals as is envisaged by CBiRC. We are also seeking to assess the CBiRC platform chemicals for a range of different sugar sources to provide insight as to the most environmentally attractive source of substrate.

Plans for the Next Year

Over the next year the LCA and techno-economic analysis efforts will begin to expand from assessment of stand-alone technologies developed by the Center to looking at the CBiRC technologies integrated into existing industrial processes and emerging biofuel/biochemical processes. We will evaluate how well the testbed technologies and any emerging new testbed technologies can be integrated into existing production processes, since such integration can improve the economic feasiblity for both processes.

We will also expand the environmental impact analysis for the testbed technologies. We will complete a generic model of greenhouse gas (GHG) emissions associated with all upstream life cycle stages as well as generic processes required in the CBiRC pathways. For example, we will complete inventories for sugar derived from sugar cane, sweet sorghum and corn.

We will also continue to revise and refine our assessments of the CBiRC testbed technologies and competing technologies, improving our analyses as the Center technologies advance. These analyses will provide improved information regarding technological feasibility and help identify research priorities associated with economic and environmental sustainability.

Expected Milestones and Deliverables

Performance milestones will again be updated in the third quarter. Further develop the use of chemical structure information as a method of screening for environmental profile of testbeds and developing LCIA impact factors. Complete by end of fourth quarter. Draft paper on QSAR methods during second quarter.

Develop draft of journal article on LCA of sugar feedstock pathways by end of fourth quarter.

Member Company Benefits

Member companies gain valuable insight into the economic and environmental viability of the Center technology through the LCA and related analyses. In particular they gain economic perspective on the prospects for the testbed technologies in both near- and longer-term. Member companies also gain a detailed understanding of the economic outlook for technologies in the individual thrusts, such as catalytic processes that have been widely reported for converting biomass-derived carbohydrates to hydrocarbons. Techno-economic analyses demonstrate the major technological hurdles in these processes and identify targets for improving these processes through research and development. These targets may represent valuable research targets for member companies interested in developing these sorts of conversion processes.

Commercialization / Technology Transfer

The LCA support area provides valuable information in support of commercialization decisions by member firms and center researchers. As we develop unique data sets, such as LCI data for biorenewable chemicals we may be able to commercialize these by distributing them as an LCI database through an organization like the ecoinvent Center which distributes the ecoinvent LCA databases, which are the mostly widely used LCI databases worldwide.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Teacher Professional Development

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University
Other Faculty: Basil Nikolau and Brent Shanks, Iowa State University
Graduate Students: Michael Nolan and Byron Upton, Iowa State University
Other Personnel: Mari Kemis, Karri Haen, Lindsey Long, and Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

CBiRC will develop a long-term partnership with the Des Moines Public School District, rural school districts in Iowa and school districts in New Mexico, to provide STEM teachers (grades K-12) with knowledge, experiences, and tools to create inquiry based learning environments in their classrooms. Emphasis will be placed on teaching general engineering concepts with a strong focus on biorenewable chemicals and fuels. Teachers will be equipped to bolster a strong sense of inquiry and curiosity for science and engineering in their students. CBiRC teachers in the Des Moines Public School District will be encouraged to work collaboratively with other teachers in their district across grades and subject areas by forming science based Professional Learning Communities (PLC's).

Project's Role in Center's Strategic Plan

Providing teachers with professional development opportunities and research experiences related to CBiRC research thrusts is a central part of the Center's educational strategic plan: to prepare a strong and diverse pipeline of students committed to continuing their college education in STEM fields.

Fundamental Barriers and Methodologies

We do not believe there are fundamental research barriers to this project.
The Research Institute for Studies in Education (RISE) uses formative and summative
The ISU Research Institute for Studies in Education (RISE) uses formative and summative assessment methodologies to evaluate the efficacy and impact of the professional development programs.

Achievements

- Twenty-four elementary school teachers from central Iowa were accepted to participate in a one-week professional development workshop *Plants in Society*. Twelve of these participants teach in the Des Moines school district. Emphasis was placed on the use of plants as a sustainable energy source. Funding for this workshop was provided in part by the NSF Plant Genome Research Program (PGRP).
- The *Plants in Society* workshop was designed to provide elementary school teachers with the motivation, confidence and resources for STEM inquiry-based curriculum and instruction development. Evaluation of the workshop showed elementary school teachers scored significantly better on a content knowledge test covering plant and biorenewables topics after participating in the five-day workshop. A follow-up assessment revealed that, compared to other professional development experiences, teachers felt more inspired to integrate new content and pedagogical techniques into their curricula when basic principles were conveyed to them as community-centered ideas such as "going green." Additionally, ninety-four percent of the teachers reported changes in their perception of the nature of science four months after completion of the program.
- Eleven middle school teachers participated in the four-week CBiRC Summer Academy to study the methods of biomass utilization to produce biorenewable products, including biofuels and bio-materials. This program was awarded $48,614 from the Iowa Power Fund to support teacher participation. Three teachers from Des Moines public schools and one teacher from New Mexico participated in the program.
- The CBiRC Summer Academy is a guided inquiry experience for secondary school science teachers, which provides the tools, experiences, and collaborative relationships necessary for translating the latest developments in STEM (science, technology, engineering, mathematics) into the classroom. During the summer experience, teachers learned laboratory techniques and basic biorenewables concepts through guided experimentation with CBiRC staff and graduate students. The program helped middle school teachers discover the nature of science in a research laboratory, and gave teachers a platform for building hands-on learning experiences in their science classrooms. Teachers gained real-world knowledge to share with their students and were able to relate scientific practices to issues in current events. More than half of the teacher reported data analyzed for the 2011 program assessment showed a significant change between pre- and post-program scores (23 of 38 matched pairs; 60.5%), including increased preparedness for teaching STEM, increased knowledge of biorenewables topics, and perceived mastery of basic laboratory skills and engineering concepts.
- With funding support from the Iowa Department of Education and in collaboration with Des Moines public school district, CBiRC hosted a two-day training for district science teachers at ISU campus. The focus of this event was to inform 25 science teachers (middle and high school) about cutting edge research in STEM areas. Teachers visited research labs of various on-gong projects including CBiRC labs.
- Eleven high school science and PLTW (Project Lead the Way) instructors participated in the CBiRC seven-week RET 2011 program including four teachers from the Des Moines public school district and two teachers from New Mexico (Albuquerque School District and Los Lunas School District). Their research projects were:

- Polyols to plastics: Development of catalysts and reaction systems.
- Effect of temperature and oxygen in the reaction environment on biochar characteristics.
- Thermo-mechanical properties of tung-oil based thermosetting polymers.
- Bio-derived block copolymers: synthesis and characterization.
- Nuclease mediated gene knockout in *Chlamydomonas*.
- Atom transfer radical polymerization of acrylated epoxidized soybean oil.
- Isoforms of 3-Methylcrotonyl-CoA Carbonoxylase of *Arabidopsis thaliana*.

- During the 2011 summer RET program, all participating teachers attended various pedagogical seminars, workshops, and discussion groups:
  - *Frontiers in Science and Engineering* weekly colloquium presented by CBiRC and other ISU faculty.
  - Two-day workshop on *Creating a Case Study based on RET research projects*. The workshop was facilitated by Dr. Prof. Clyde Herreid, Director, National Center for Case Study Teaching In Science, University of Buffalo, NY.
  - Weekly learning group discussions centered on case study development facilitated by two master teachers.
- Evaluation of the RET program produced the following outcomes:
  - Teachers expanded their knowledge of approaches to integrate collaborative inquiry-based activities in their classrooms and learned the value of keeping scientific course content current.
  - Teachers engaged in critical thinking about their teaching philosophies and methods and their impact on students' learning, including learning pedagogical methods directed by different learning styles that recognize learners as unique individuals.
  - Teachers gained collaborative relationships with other high school, middle school and university instructors to foster their continued growth in the realm of science education.
  - Teachers' teaching philosophies were particularly influenced regarding the methods used to engage students in the classroom.
  - Teachers planned to incorporate (a) hypothesis-driven lab exercises and discussions, (b) critical thinking activities, (c) long-term experiments to engage students in continuous active learning, and (d) the development of analytical skills in their classroom curricula.
  - Participants gained laboratory skills, a better understanding of scientific inquiry, persistence and patience in the laboratory setting, and confidence working in a research environment.
  - Participants gained a better understanding of biorenewables and CBiRC's research goals.
- Selected teacher quotes relating to the summer programs:
  - *Our content knowledge has been improved and I think that we have a better understanding of how we teach engineering and what engineering actually is. [I'm confident that] if the student asks me what material engineers do or what a chemical engineer does, I can give them a better understanding of what it is.*

- *I think it's good to experience not knowing how to do everything and it helps remind yourself of what your students are going through a little bit. They don't have all the answers and the thinking processes that they need to put things together and there is a gap between what you know and they know. [The RET helps you to] experience that from the other side. It gives you empathy and patience and the ability to communicate what you're doing to the students better.*
- *I think that just getting kids to realize that science is just as much a process as it is a right answer or getting the right answer. So much of science is just day in and day out grunt work, and you have to stay constant with it, you've got to stay diligent.*
- *Sometimes our kids think of science as being the nerd, the geek, the brainy kids. Some people have different level of science ability based on what they were doing, and I think that's important for us this to bring across to our students. You don't need to have a 34 to 36 on the ACT to still get into science and do it.*
- *My lab experience really deepened my content knowledge. It's just a very small part of chemistry but to actually have to think about it and understand it well enough to explain it, really made me get in depth. It actually cleared up a couple misconceptions I had.*

Other Relevant Work

- RET participants are implementing the case studies they developed during their summer professional development. Teachers have stated the case study technique was an easy way to translate the CBiRC research experience to the classroom.
- RET teachers participated in a philosophy of science study that included the Views on Science and Education (VOSE) questionnaire. The results will be used for continuing research and publication in the area of teacher perceptions of the nature of science.
- RET participants are encouraged to bring their students to visit CBiRC at the ISU campus.

Plans for the Next Year

- As a follow-up to the Plants in Society workshop, CBiRC will offer a one-week workshop for elementary school teachers on Biorenewables in summer 2012.
- Measure student impact as a result of teacher participation in the RET program.
- Conduct follow-up surveys with the teachers who participate in the professional development programs to determine the program effects on their teaching and their students learning during the ensuing academic year.
- CBiRC will extend the RET program to include more participants from rural Iowa and New Mexico as well as teachers from the Des Moines Public School District.

Expected Milestones and Deliverables

1. Poster presentation at NSF Engineering Education Awardees Conference, March, 2012. Title: NSF Engineering Research Center for Biorenewable Chemicals Pre-College Education Program.
2. Article accepted with revision by the Journal of Science Teacher Education: *Elementary School Science for a More Sustainable Future* by Karri M. Haen, Mari Kemis, Lynne Bleeker,

Jonathan Wendel and Adah Leshem.

3. Presentation of CBiRC's Summer Academy for Middle School Teachers submitted to the National Science Teachers Association, NSTA, STEM Forum and Expo conference, May 2012.

Member Company Benefits

The middle and high school teachers who participate in CBiRC professional development programs will be able to help their students better understand career opportunities in the areas of engineering and biorenewables and will hopefully attract students to become future scientists, engineers, and potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Pre-College Education Modules

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University

Other Faculty: D. Raj Raman and Laura Jarboe, Iowa State University

Other Personnel: Karri Haen, Mari Kemis, and Lindsey Long, Iowa State University

Statement of Project Goals

CBiRC will develop, in collaboration with partnering schools and teachers, three inquiry-based learning modules for use in grades 6-12 that will introduce students to the value of biorenewables and engineering concepts.

Project's Role in Center's Strategic Plan

The education modules will engage pre-college students in the fields of engineering and biorenewables.

Fundamental Barriers and Methodologies

We do not believe there are any fundamental barriers to this project. The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.

Achievements

- Work towards this goal began during the training workshop for the 2009 summer RET program. Teachers piloted two classroom activities: ethanol production and genetic engineering. In Summer of 2010 the modules were further developed by RET participants and GK-12 graduate students.
- During the past year (2011-2012), the modules were further developed in collaboration with the ISU Office of Biotechnology, CBiRC faculty, CBiRC lead teachers and GK12 graduate students and their middle school teach partners. Efforts were focused on the revision and modification of the ethanol activity with the addition of two more topics: biodiesel production and analysis of corn structure. Three inquiry-based curriculum units were developed and made available on the Internet: http://www.biotech.iastate.edu/publications/BiorenewablesCurriculum/.
- Over 500 students, ranging from middle school to graduate students, used the biodiesel module in their classrooms or as an outreach activity. We successfully implemented the biodiesel module in 4 CBiRC partner middle schools in Des Moines, IA. Middle school student made

soap as a bi-product of the experiment and used this soap to wash their hands in class.

- Equipment and materials are available via the Office of Biotechnology loan program.
- The Iowa State University BioEconomy Institute provided $5,000 to support this effort.

Other Relevant Work

- The biorenewable learning modules will be presented as part of a presentation at the National Science Teachers Association (NSTA) STEM Forum and Expo in May 2012.
- Data are being collected to determine the impact these activities are having on students who conduct these curriculum modules.

Plans for the Next Year

The education modules will be included in more classrooms in the Des Moines school district. Workshops will be conducted during the summer professional development programs to train teachers and graduate students so that the modules can be effectively implemented in the classrooms.

Expected Milestones and Deliverables

1. Three education modules will continue to be evaluated in middle school and high school classrooms during the 2012-2013 academic year.
2. An additional education module will be developed.
3. The education modules will be demonstrated during teacher professional development workshops and GK12 training workshops.

Member Company Benefits

Providing K-12 students and teachers with educational materials associated with biorenewables will help students better understand career opportunities in the area of biorenewables and will hopefully attract students to become potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Young Engineers & Scientists

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University

Other Faculty: Joe Noel, Salk Insitute for Biological Studies

Other Personnel: Mari Kemis, Karri Haen, and Lindsey Long, Iowa State University; Kate Woods, Salk Insitute for Biological Studies

Statement of Project Goals

- Provide 10^{th}-12^{th} grade high school students the opportunity to participate in CBiRC related research projects as well as non-associated engineering and scientific research projects.
- Provide pre-college students exposure to both academic and career options in science, technology and engineering fields.

Project's Role in Center's Strategic Plan

Participation in CBiRC-related research projects will engage pre-college students in the fields of engineering and biorenewables.

Fundamental Barriers and Methodologies

We do not believe there are any fundamental barriers to this project. The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.

Achievements

- The CBiRC Young Engineers program has expanded and currently offers research internship opportunities to 10^{th}-12^{th} grade high school students in the physical and life sciences as well as non-CBiRC associated fields of engineering. The program is now called *Young Engineers and Scientists* (YES) and is offered year round. Students who participate in the fall and spring semesters are typically from local high schools and do not receive stipends but instead receive high school credit listed as "independent research study" on their high school transcript. Students who participate in the summer YES program are recruited from ISU's Science Bound program that works closely with the Des Moines and other central Iowa school districts' underrepresented minority students to encourage and support their preparation for and pursuit of an academic degree in STEM. These students receive a stipend but do not receive credit. All students complete periodic assessment surveys. All students prepare and present a poster

outlining their research project. Over the past year 22 high school students participated in the program; 4 of these participated in the summer program and were recruited from Science Bound.

- All summer 2010 YES participants (recruited from Science Bound) are currently enrolled at ISU; one is majoring in biosystems engineering, a second in kinesiology, and the third in biology. The fourth student elected not to continue in STEM.
- One 2009 YES participant who was under the mentorship of Dr. Laura Jarboe is now majoring in engineering at Rice University.
- In summer 2011, CBiRC continued its collaboration with ISU's Science Bound program to recruit three rising seniors (all underrepresented minority students) from Des Moines high schools and one rising junior from Marshalltown (IA) High School. Science Bound works closely with the Des Moines and Marshalltown School Districts" underrepresented minority students to encourage and support their preparation for and pursuit of an academic degree in STEM. These four students participated in the summer YES program.
- Two high school students participated in the YES program at the Salk Institute, one of CBiRC's partner institutions.
- Program evaluation findings show that students participating in the program have:
    - A deeper appreciation for science and scientists.
    - An understanding that science is done by 'common' people.
    - Self-confidence in their ability to conduct research.
    - Knowledge of different fields of science.
    - A better understanding of academic options.
    - A stronger interest in pursuing a research career.
- Student comments provide insight into their experiences:
    - *I wasn't really sure what to expect because I wasn't sure what all we were going to be doing but it was a lot more. In my lab, I was treated like as equal as everyone else.*
    - *My experience in this program solidified what I want to do. I was always wavering on whether chemistry was a good idea for me or not but now that I know, it helps me look for colleges that would have strong programs in chemistry. I think it just adds to the well-roundedness of a person that they did something over their summer.*
    - *For me it was a good experience. I really wanted to be in mechanical engineering, and this actually gave me a little key, or a little place, a little certain thing in mechanical engineering, which is a really broad subject, so it really got me interested. Just being here on campus, [let me] see how things work out and how being with a mentor, grad students, undergrads, and my professor really gives me the feeling that I could do this one day.*
    - *It's not like anything I really knew about and it opened up my opinions to other things that I could do in the future. I think it helps us get into college because they'll see that you've done something or had experience ahead of time, and it's just like you know what you're doing.*

Other Relevant Work

The Young Engineers & Scientists program has formally established two tracks: an Academic Year Experience and Summer Internships. By distinguishing each track, we are able to provide more students an opportunity to gain hands-on research experience to pursue ASTEM (Agriculture, Science, Technology, Engineering, and Mathematics) degrees and assist more faculty with their broader impact efforts in K-12 education.

Plans for the Next Year

- CBiRC will continue the Young Engineers & Scientists program to offer high school students research internship opportunities.
- CBiRC will continue to collaborate with Science Bound, Iowa State University's premier pre-college program for underrepresented minorities, to increase the number of ethnically diverse Iowa students who pursue ASTEM degrees.
- CBiRC faculty at partner institutions will be encouraged to mentor high school students as part of the CBiRC YES program.

Expected Milestones and Deliverables

1. The high school students who participated in the Young Engineers & Scientists program at Iowa State University will present posters outlining their research projects at a poster receptions either at Ames High School in May 2012 or at the conclusion of the 2012 summer RET program.
2. A number of the students will present at state Science Fairs.

Member Company Benefits

Providing pre-college students exposure to how research is conducted in the field of engineering will help students better understand career opportunities in this area and will hopefully attract students to become potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: *Symbi*, Iowa's GK-12 Program: Growing Iowa's Scientists for a Greener Tomorrow *(a sponsored project)*

Thrust: Pre-College Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Adah Leshem	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Adah Leshem, Iowa State University

Other Faculty: Basil Nikolau, D. Raj Raman, Iowa State University

Graduate Students: Bryon Upton, Iowa State University.

Other Personnel: Karri Haen, Mari Kemis, Lindsey Long, Diana Loutsch, Iowa State University

Statement of Project Goals

Engage graduate students conducting interdisciplinary research in the area of biorenewables, with Des Moines, IA, middle school educators, students and their parents, and administrators. The objectives of this engagement are to: 1) provide graduate students with the skill sets and communication proficiency to explain their science and illustrate core STEM principles to a young and receptive audience; and 2) provide middle school students exposure to inquiry-based learning experiences and authentic demonstrations of mastery of core concepts.

Project's Role in Center's Strategic Plan

- Provide graduate students and K-12 teachers with professional development opportunities, specifically to become better communicators of STEM subjects.
- Provide pre-college students with exposure to CBiRC and the value of biorenewables and engineering concepts.

Fundamental Barriers and Methodologies

We do not believe there are any fundamental barriers to this project. The ISU Research Institute for Studies in Education (RISE) uses formative and summative assessment methodologies to evaluate the efficacy and impact of the program on GK-12 Fellows, middle school teachers, and middle school students.

Achievements

- CBiRC was awarded Iowa's first GK12 grant in May 2010.
- Iowa's first GK12 program was branded with the name *Symbi* and a website was developed: **www.gk12.iastate.edu/**
- To date 12 STEM graduate students have filled the position of GK12 Fellow (two graduate students have repeated the program).

- To date 8 science teachers from five middle schools and one high school in the Des Moines school district, have participated in the Symbi program as GK-12 teachers, impacting approximately over 2,000 students. Four of these schools are minority-serving institutions.
- In May and April 2011 Symbi hosted Science Days at three middle schools in Des Moines. These events took place in the schools' gym and featured on-going presentations and demonstrations by ISU STEM graduates students and local industry. All the students in these schools spent their science class period visiting with the presenters to learn more about different subject areas in STEM and possible STEM career paths. Over 1,000 students participated in Symbi Science Days.
- The Fellows and Teachers completed training in Summer 2011 in preparation for classroom collaboration and attended the graduate course *Symbi Professional Practices Tutorial*, CI 593A, taught by Dr. Denise Crawford in the ISU Dept. of Curriculum and Instruction.
- To assess and improve the communication skills of the Fellows, ISU Department of English professor Amy Slagell presented a workshop on communication effectiveness in the classroom. A doctoral level graduate student in Technical Communications, Department of English at ISU, was hired to observe the Fellows in the classroom and provide individualized and group feedback about how to improve classroom communication effectiveness, specifically with regard to science and engineering topics covered in the classroom.
- Monthly surveys of the Fellows and Teachers indicate that *Symbi* is meeting all project objectives. Middle and high school students are very receptive to the presence of the graduate student "resident scientists/engineer" in their classrooms. "A resident scientist brings authenticity to the science classroom where students come face to face with the nature of science and explore career opportunities. This consistent presence provides a face to the research community and allows students to identify with, and see themselves as scientists/engineers." (GK12 teacher comments).

  Symbi Teachers report that their students are more engaged and are asking more questions than in previous years. *Symbi* Teachers also report that the graduate students have demonstrated improved communication skills over the past semester. "Our resident engineer does a nice job of rephrasing scientific language into language students better understand…. The resident scientist brings more experience and education into the classroom for the students to learn from…. Students are asking more relevant questions and including more details in their writing" (GK12 teacher comments).
- Practiced lesson plans and projects have been developed as a result of the Fellow and Teacher collaboration in the classroom. These lesson plans and projects are posted on the *Symbi* website and available to the public to print and implement into their classroom: http://www.gk12.iastate.edu/classroom_projects/lesson_plans.asp

Other Relevant Work

- Data are being collected from middle/high school students, Fellows, and Teachers to determine the impact *Symbi* is having on middle school students. At this time, data have been collected from approximately 800 students (Fall 2010 and Spring 2011), with 383 matched responses. Data were collected from over 900 students in Fall 2011, including at least 150 students who have matched data from the previous year (meaning that they have been in classes with more than one GK12 Fellow and Teacher).

- Eighth grade students in classes with a GK12 Fellow (who responded to both the pre- and post-Middle School Science Surveys in 2010-11) were significantly more likely to have higher interest in problem solving and engineering. Additionally, they report a significantly higher level of agreement that (1) science and engineering can help solve world problems, (2) their science classes make them more interested in going into science of engineering as a career, and (3) they are confident that they will be successful if they pursue a career in science or engineering. The students also were less likely to want the teacher to give them answers to science problems, which is related to the efforts of the Fellows and Teachers to introduce experimental science in the classroom.
- Student attitude data toward science and science as a career have also been collected during early Fall semester 2011 from Hoover High School (Des Moines, IA) science students and will be collected as a post-survey in April 2012. It is hypothesized that student attitudes and career aspirations can be significantly impacted in classrooms with resident scientists and/or teachers who have had specific professional development in inquiry-based learning and laboratory experience. Changes in attitude and career aspiration will be analyzed by three groups—students with a GK12 Fellow as resident scientist in the classroom, students with an RET teacher, and a control classroom (no resident scientist and no or limited professional development). Analysis and continuing data collection will take place during Year 3 of the GK12.

Plans for the Next Year

- Select a total of nine graduate students each year to become *Symbi* Fellows.
- Select a total of nine middle school teachers each year to partner with the *Symbi* Fellows.
- Improve GK-12 training program based on evaluations from 2011-2012 teachers and fellows.
- Plan and implement a *Symbi Science Day* at three partner middle schools in Des Moines to create an event that will provide all students, approximately 2000, to learn about ASTEM (Agriculture, Science, Technology, and Mathematics) research and careers.
- Continued data collection to examine middle school student achievement, attitudes toward science, and career plans in STEM longitudinally.

Expected Milestones and Deliverables

1. Continued growth of the *Symbi* program with more teachers, middle/high school students, and graduate students involved.
2. Greater involvement with the Des Moines community through events like Science Days.
3. Increased participation of *Symbi* fellows and teachers at Annual GK-12 meetings.
4. Further development of the Symbi lesson plans and projects will be used in other CBiRC programs and made available to the all CBiRC teachers as a way to directly involve their students in hands-on learning and discovery activities.

Member Company Benefits

Symbi will invite member companies to exhibit their products at Symbi Science Days. Symbi Fellows will accompany the middle school students during the school science days to help them better understand career opportunities in the area of biorenewables and will hopefully attract students to become potential employees for member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: CBiRC Graduate Minor & Graduate Certificate (for extension to partners)

Thrust: University Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Dave Raj Raman	**03/04/12**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Raj Raman, Iowa State University

Other Faculty: Brent Shanks, Iowa State University

Other Personnel: MaryAnn Moore, Karri Haen, and Peter Keeling, Iowa State University

Statement of Project Goals

The minor in Biorenewable Chemicals allows students from a variety of allied disciplines to understand the opportunities for developing biorenewable chemicals via a combination of biocatalytic and chemical catalysis steps. In addition, students in the minor get explicit entrepreneurial internship training, a background in the general issues related to production and processing of biorenewable resources, and exposure to the economic and environmental realities of the chemical industry. The interdepartmental minor resides within the Graduate College and is formally affiliated with CBiRC. The minor consists of a 14-credit hour sequence: 8 hours of graduate coursework encompassing *Fundamentals of Biorenewable Resources and Technology* (3 cr), *Biological and Chemical Catalysis* (3 cr), *The Evolving Chemical Industry* (1 cr), and *Entrepreneurship in Biorenewable Chemicals* (1 cr), plus 6 credits of coursework selected from a list of courses reflecting CBiRC's three technical thrust areas:

- Thrust 1: New Biocatalysts for Pathway Engineering
- Thrust 2: Microbial Metabolic Engineering
- Thrust 3: Chemical Catalyst Design

Additional training of students in the graduate minor occurs through the annual CBiRC center-wide meeting where students will present posters and learn about each other's research findings, and thereby gain a better appreciation for both chemical and biological catalysis routes for producing biorenewable chemicals.

Because CBiRC partner institutions lack the faculty numbers (and institutional structures in some cases) needed to institute stand-alone graduate minor programs, a Graduate Certificate in Biorenewable Chemicals program was also instituted.

The disciplines of biological and chemical catalysis have traditionally been separate. And while some of this separation will always exist, the core mission of the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) is to transform the chemical industry by integrating biological and chemical catalysis systems to create a generalized framework for producing biorenewable chemicals. Graduate education is central to achievement of this

mission, because graduate students will develop the expertise needed to drive future research programs in this area, both in academic and industrial settings. The minor and certificate furthers CBiRC's mission by producing disciplinary experts from programs like Chemical Engineering, Chemistry, and Biochemistry, Biophysics, and Molecular Biology, who are interdisciplinary trained to become globally-competitive college graduates capable of designing integrated chemical/biological processing systems.

Project's Role in Center's Strategic Plan

The minor and certificate programs are central to CBiRC's strategic plan to educate graduate students in this area.

Fundamental Barriers and Methodologies

Declaring a minor at Iowa State University required the approval by all departments or sponsoring groups (five curriculum committees, five department heads), the appropriate college curriculum committees (two), the college faculty for one of the colleges, the college deans (two), the Faculty Senate Curriculum Sub-Committee, the Dean of the Graduate College, and the Executive Vice President and Provost. We have surmounted this barrier, but have a second, deeper barrier, which is that talented graduate students (and their CBiRC-affiliated major professors) may feel that the return on investment (of student time, associated with additional coursework need for the minor) is not sufficiently large, and the program may fail to have a significant number of students.

Although mechanisms exist to offer all of the four core courses to students at all partner institutions, graduate minor degrees cannot be conferred by ISU to non ISU students at the partner institutions. So a major barrier to making this graduate education available to **all CBiRC graduate students**, and not just those at ISU, is the non-transferability of the graduate minor. To overcome this barrier, we are making a Graduate Certificate available to all non-ISU CBiRC graduate students. The coursework requirements are identical (8 credits of core coursework, taken via distance methods) plus six semester hour equivalents of thrust-specific courses, taken at the home (i.e., partner) institution. The certificate, which does not carry a university seal, is granted by CBiRC, and consists of a formal "certificate" and a letter from the CBiRC University Education Program Director, detailing the coursework taken to achieve the certificate, and the learning objectives of the program. The latter document can then be used by students as part of their applications for jobs.

Achievements

Since the initiation of the graduate minor program, all of the CBiRC core courses have been taught at least once. *Catalysis and catalytic processes*, BR C 688, focused on the fundamentals of hetero-geneous and bio-catalyst synthesis, characterization and reaction testing, was first offered in the spring of 2010. Nineteen students from three universities (ISU, the University of Virginia, and the University of New Mexico), participated in the course. Of these, nine students were affiliated with CBiRC. Students commented that they found both the broad overview and particulars of the course (derivation of equations, specific research examples from the literature, industrial applications, etc.) very pertinent to their research and potential careers.

BR C 506, *The Evolving Chemical Industry*, was offered during the summer of 2010 with distance education opportunities for students at partner universities. The course was designed in order to help students gain an understanding of the current chemical industry and its development, with special emphasis on the commercialization process of biorenewable chemicals. Seventeen students

from CBiRC partner institutions were enrolled in the course: 15 from ISU and two from other universities. Evaluations showed students felt very strongly that this course helped them gain an understanding of the importance of economic and environmental constraints in the practice of engineering.

Additionally, the graduate minor program offered a new 1 credit course in spring 2011 (and again in spring 2012), BR C 507, *Entrepreneurship in Biorenewable Chemicals*. This course was designed to develop an understanding of discovery research and its relationship to entrepreneurship and innovation in the broad area of biorenewables. Participants of the course will understand the critical importance of developing a sound techno-commercial analysis and evaluation of intellectual property, as well as learn how to utilize local resources in entrepreneurship. The course objectives include teaching students how to define key assets, write a business plan, and how to take the necessary steps to go about founding a company and securing research funds.

Other Relevant Work

We are unaware of any other graduate programs in biorenewable chemicals.

Methods developed as part of a USDA Higher Education Challenge Grant to develop a Virtual Education Center in Biorenewable Resources (PI: Raman) are heavily used in CBiRC's graduate minor efforts. Specifically, the USDA project Virtual Education Center model relies upon sharing video lectures – rather than the onerous moving of student credit hours across institutions – to allow instructors at multiple sites to contribute their expertise to a course. In the case of the Graduate Minor in Biorenewable Chemicals, all four of the core courses are using this model, with additional lectures from Distinguished Regents Professors Abhaya Datye (University of New Mexico) and Earnest Jackson Oglesby Professor Bob Davis (University of Virginia).

Plans for the Next Year

Oversee and grow both the minor and certificate in Biorenewable Chemicals. Use pre- and post-surveys to understand how effectively the courses are addressing our hypothesis regarding the training of creative, adaptive, engineers.

Expected Milestones and Deliverables

Over the next five years, at least 15 PhDs will graduate with a minor or certificate in Biorenewable Chemicals. Both programs currently enroll a handful of students.

Member Company Benefits

The graduate minor is the culmination of CBiRC's educational mission, and the part of the educational programs most likely to *directly* impact member companies by training outstanding engineers (and, in CBiRC's case, scientists) who will be employed as interns or permanent employees at member companies.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Research Experience for Undergraduate (REU) Program

Thrust: University Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Dave Raj Raman	**03/05/12**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: D. Raj Raman, Iowa State University

Other Faculty: Laura Jarboe, George Kraus, Basil Nikolau, Peter Reilly, Brent Shanks, Jackie Shanks, Derrick Rollins, Eve Wurtele, and Keith Woo, Iowa State University; Rob Anex and Jim Dumesic, University of Wisconsin; Nancy Da Silva and Suzanne Sandmeyer, University of California – Irvine; Joseph Noel, Salk Institute for Biological Studies

Graduate Students: Jason Anderson, Jonathan Beasley, David Cantu, Shivani Garg, Ping Liu, Gina Roberts, Mark Stenerson, Lucas Showman, Quyen Truong, and Jong Moon Yoon, Iowa State University; Thomas Schwartz, University of Wisconsin; Javier Cardenas and Tarek Najdi, University of California – Irvine

Other Personnel: MaryAnn Moore, Karri Haen, Mari Kemis, and Marna Yandeau-Nelson, Iowa State University; Kate Woods, Salk Institute for Biological Studies

Statement of Project Goals

The CBiRC REU program strives to recruit, nurture, and train the next generation of creative and adaptive engineers who will be capable of bridging the gap between chemical and biological catalysis. In so doing, we hope to produce technical professionals capable of moving the US chemical industry toward a more sustainable model of production based on biorenewable feedstocks though experiential learning in CBiRC faculty laboratories. During the course of the CBiRC REU program, opportunities exist for student specialization of research in one or a combination of CBiRC thrust areas: biocatalysts for pathway engineering, microbial metabolic engineering, chemical catalyst design, or life cycle analysis of biorenewable chemicals. The program further integrates hands-on research with a series of weekly lectures or other center-wide interactions, which include lab tours, workshops, and meetings, and the opportunity to present student project results to the CBiRC community. CBiRC educational programs strive to instill students with a multidisciplinary background, so that they can devise creative approaches to solving engineering problems, including recognizing the wide-ranging potential for both chemical and biological catalysis for the production of environmentally sustainable chemicals. Further, students are engendered with the understanding that economic, environmental, and ethical constraints are central to the practice of engineering, and, thus, CBiRC engineers should be capable of evaluating their work based upon these criteria.

Project's Role in Center's Strategic Plan

The Research Experiences for Undergraduates program (REU) is a major component of the CBiRC university education program, which particularly focuses on undergraduate student training. Through participation in the REU program, science and engineering students develop their skills through experiential learning with the Center's interdisciplinary research. Guided by CBiRC faculty, postdoctoral research associates, and graduate students, undergraduates become a part of a team involved in the development of new integrated catalytic systems for the conversion of bio-based feedstocks to industrial chemicals.

Fundamental Barriers and Methodologies

Due to the nature of this project, there are not fundamental research barriers per se. However, there are challenges associated with the establishment of large programs that serve multiple universities. Since the summer of 2010, the CBiRC REU has grown to be a multi-institutional program, thus creating logistical challenges associated with the coordination of students and programs among the participating universities. Some of these challenges are straightforward, such as obtaining IRB approvals for continued evaluation of CBiRC-affiliated undergraduate students. Other issues are implicit to any program that has partners which do not exist locally. Extending the program to partners has encouraged us to determine how to better coordinate laboratory safety training, general program orientations, and other center-specific meetings, such that students do not have to participate in these exercises more than once. All of these issues were addressed in 2010 and again in 2011. In 2010, although we had an academically strong cohort, friction between two REU participants and each other, as well as with their host labs, caused a general frustration in the group that was difficult to overcome. In 2011, we instituted pre-offer interviews of all students, in which personal interaction styles were probed. Furthermore, during interviews and again during orientation, the importance of collegiality in host labs and between REUs was emphasized. Due to these actions (and perhaps also through fortuitous selection), the 2011 REU was by far the most successful one to date. We are hoping to repeat this in 2012.

Plans for the Next Year

Over the next four years the CBiRC REU program will graduate an additional 50 students (for a total of 80+ by March 2016), with 15 – 20% of these students having spent the majority of their summers at partner institutions. The progress of these 80+ graduates will be monitored to the best of our ability, and we expect that at least 40 of our graduates will go on to graduate school, with half of those going to fields relevant to biorenewable chemicals. CBiRC REU graduates have already gone on to graduate programs in chemistry, with the intent to work on biorenewable chemicals. We will continue our aggressive efforts to recruit from under-represented populations but due to comments from the 3rd year site visit team will no longer partner with ISU's innovative SPEED (Summer Program for Enhancing Engineering Development) program. Recruitment processes included (a) advertising the program among CBiRC member institutions, (b) sending invitations to faculty mentors at minority serving institutions and underrepresented minority students who participated in recruitment activities at ISU, (c) promoting the program through the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers, and (d) listing the program description on the National Science Foundation website. We will report on the successes and challenges of this geographically and disciplinary diverse REU program in refereed publications and presentations at national meetings.

Expected Milestones and Deliverables

Of the 14 students anticipated for the 2012 REU program, we expect to have 10 of those students remain at Iowa State University throughout different research projects and 3-5 students attending partner institutions after they complete orientation at Iowa State. They will work on interdisciplinary teams with faculty, graduate students, post-docs, and in some cases industrial partners. They will also engage with students participating in other Iowa State University based REU program in seminars, short courses, research tours, field trips and social events with mentors, graduate students, postdoctoral associates and others involved in the research of biorenewables. Students participating in the CBiRC REU program will be expected to work in a research lab for 40 hours per week for 10 weeks, participate in weekly lab meetings and all other scheduled events. At the end of the program they will be required to present their research findings both orally and in the form of a poster.

Member Company Benefits

Potential exposure to creative and adaptive engineers capable of bridging the gap between chemical and biological catalysis.

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Iowa State Coleman Faculty Entrepreneurship Fellow (*a sponsored project*)

Thrust: University Education Program and Innovation Ecosystem

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Peter L Keeling	**02/29/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Peter Keeling, Iowa State University

Other Faculty: D. Raj Raman, Iowa State University

Statement of Project Goals

The project goal was to create and develop a course in Technology-Led Entrepreneurship related to Biorenewable Chemicals. This was done within the framework of the Coleman Fellows program in order to learn from other experiences in teaching and fostering entrepreneurship in academia. A fundamental mission of the course is to show that entrepreneurship is not the sole realm of business school graduates. Instead technology-led entrepreneurship is shown to be a fundamental skill that all scientists and engineers should strive to acquire.

Project's Role in Center's Strategic Plan

The Entrepreneurship Course was designed as an addition to the CBiRC Graduate Minor in Biorenewable Chemicals. The course contributes to the Center's strategic plan by developing an improved understanding of entrepreneurship at the graduate student level. The Graduate Minor allows students from a variety of allied disciplines to understand the opportunities for developing biorenewable chemicals via a combination of biocatalytic and chemical catalysis steps. In addition, students in the minor gain explicit entrepreneurship experience, a background in the general issues related to production and processing of biorenewable resources and exposure to the economic and environmental realities of the chemical industry.

Fundamental Barriers and Methodologies

The Entrepreneurship Course program overcomes traditional barriers and misconceptions that entrepreneurship is difficult and only for wealthy extravert superstar entrepreneurs. The course impacts students by teaching them that entrepreneurship is just as much a part of existing businesses and academia as it is about forming a startup entity. The course impacts the Center by forming a foundation of understanding of the principles of entrepreneurial practices and how this must be firmly embedded in technical and commercial analyses of any new innovation or biorenewables opportunity. The course introduces graduate students to discovery research and how this is evaluated as a risk-reward equation. It is expected that this will have a trickle-effect into the CBiRC faculty. The course shows how new knowledge leads to innovations and inventions that must secure sustained funding to become valuable. It introduces students to the process of going about seeking initial funding from various sources including the SBIR program as well as State

opportunities, Angel funds and Venture Capital funds. This process is demonstrated to be fundamentally similar to the process of project evaluation in large companies. The course proceeds with a step-by-step review of how to put together professional business/project plans and presentations in order to position a startup entity or new project for sustained ongoing funding.

Achievements

In 2011, the course had 15 graduate students. All students completed the course and four of them went-on to found a company. Two students merged to form a joint venture (RecyclaR) and two students each pursued their own vision to found Abios Pharmaceuticals and OmegaChem. The last entity just won an I-Corps grant which will start in March, 2012.

Other Relevant Work

There are many types and levels of entrepreneurship that are traditionally designed within the university business school curriculum. The CBiRC course focuses on Technology-Led Entrepreneurship and is a fundamental and vital departure from this traditional business-led realm.

Plans for the Next Year

The course is planned to continue indefinitely within the Graduate Minor in Biorenewable Chemicals. In 2012, the course became a formal course requirement for another graduate minor entitled “Biorenewable Resources and Technology”.

Expected Milestones and Deliverables

The course stimulates and nurtures an entrepreneurial spirit within CBiRC. We hope we can stimulate at least 2 new startup companies each year.

Member Company Benefits

Industry members are inherently risk-averse. Yet they can gain from this, because startup entities are better able to deal with risk. They can become sources of funding and become strategic mentors as well as customers. The industry members could eventually become owners of these startup entities.

Commercialization / Technology Transfer

Translational Research Grants are a superb source of funding for early stage startup entities. Creating startup entities is a fundamental metric of the Gen-3 ERC’s.

NSF Engineering Research Center for Biorenewable Chemicals

Project Summary

The following is actually an associated project. However, for Gen-3 ERC's, foreign partner associated projects may include a project summary rather than only an abstract if the project is of particular importance to the center's research and/or education strategic plan.

Project Title: PIRE: Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials

Thrust: International Education Program

Prepared By:	Date *(in U.S. date format)*:	Reporting Period:
Abhaya K. Datye	**02/17/2012**	**03/01/2011 to 02/29/2012**

ERC Team Members

Project Leader: Abhaya K. Datye (U-NM)

Other Faculty Investigators: Brent Shanks, Rich Larock and George Kraus (ISU); James A. Dumesic (U-WI); Matthew Neurock and Robert Davis (U-VA); Robert Schloegl, Mathias Scheffler and Malte Behrens (Fritz Haber Institute of the Max Planck Society); Markus Antonietti (Max Planck Institute for Colloids and Interfaces), Ib Chorkendorff and Thomas Hansen (Technical University of Denmark) Stig Helveg and Esben Taarning (Haldor Topsoe A/S), Hans Neimantsverdreit and Peter Thune (Technical University Eindhoven, Netherlands), Bert Weckhuysen, Krijn de Jong and Harry Bitter (Utrecht University, Netherlands) Leon Lefferts (University of Twente) and Dmitry Murzin (Abo Akademi, Finland).

Post-doctoral Associates: Barr Halevi and Hien Pham (UNM)

Graduate Students: Ulises Martinez, Andrew De La Riva, Angelica Sanchez, Jonathan Paiz, Eric Petersen and Tyne Johns (UNM); Juan Lopez-Ruiz, Matthew Ide, Joe Kozlowski, Oliver Daniel, David Hibbitts, Craig Plaisance and Sara Davis (UVA); Pedro Ortiz Toral, Ryan Snell, Michael Nolan, Thomas Garrison, Jason Anderson, Keenan Deutsch and Jennifer Lee (ISU); Elif Gorbuz, Drew Baden, Yomaira Pagan Torres and Carrie Ferberow(U-WI)

Undergraduate Students: Valerie Ashbacher (UNM), Elliott Combs (ISU)

Statement of Project Goals

This Partnership for International Research and Education (PIRE) brings together together four U.S. and eight European institutions to investigate critical steps required for chemical transformation of biomass-derived reactants into useful products. The five year plan for collaborative research focuses on metal-catalyzed conversion of carbohydrates and their derivatives to chemicals, fuels and materials. The educational aspects of the collaboration draw upon the shared intellectual and physical resources of each partner to provide multi-faceted international experiences for U.S. graduate and undergraduate students and post-docs. The resulting internationally distributed, virtual center helps prepare a new generation of globally-engaged science and engineers while the research partners pursue compelling research questions associated with biomass conversion and enhanced

engineering of metal catalyzed reactions.

The PIRE network brings together complementary strengths, for instance the U.S. partners specialize in aqueous phase processing, microkinetic modeling, and kinetic and mechanistic characterization of catalysts. The German counterparts are well known for novel catalyst synthesis and modeling of chemical reactions. Danish partners bring strengths in surface science approaches to studying new catalysts and theoretical expertise in modeling catalytic reactions. The groups in Netherlands are well known for development of in-situ spectroscopic techniques and Finland is known for its research on wood chemistry, due the plentiful supplies of woody biomass. Together, the University of New Mexico-led PIRE team will work to achieve conversion of specific C-C or C-O bonds in the presence of multiple similar functional groups and to improve our understanding of:

1) adsorption of molecules with a high level of functionality on metal surfaces;
2) the role of water or solvent in liquid phase processing; and
3) how to build in hydrothermal stability into catalysts.

The results will lead to innovative molecular engineering for conversion of biomass-derived reactants to fuels, chemicals and materials.

Sustainable production of chemicals, materials and energy from renewable resources provides a rich source of research problems that can be integrated with the education of students participating in PIRE activities. This model includes international mentoring, research internships and summer research for U.S. graduate and undergraduate students, as well as summer schools and course development. Overall, results stemming from this PIRE should fulfill the program objectives of building international partnerships that advance research and provide innovative educational opportunities through valuable contributions to future engineering in the areas of biomass conversion, sustainable energy and renewable resource development.

Project's Role in Center's Strategic Plan

The project is most directly tied to Thrust 3 in the Center's strategic plan and allows us to bring in new capabilities not possible through our US network of partners. The international experiences for students and the ability to work in large international teams will be an important component of the ERC strategic plan.

Fundamental Barriers and Methodologies

In establishing a large collaborative network, the first barrier is to get the partners to learn about the complementary expertise and how to integrate it into their projects. The US team members are actively working with each other, and the collaborations with the EU partners are growing. We have had changes in our collaborators in Denmark; Prof. Claus Christensen moved from the Danish Technical University to Haldor Topsoe and then left Haldor Topsoe; Rafal Dunin Borokowski left DTU for Germany, but we have now established ties with Esben Taarning at HTAS and with Thomas Hansen at DTU. The research focus is on understanding bimetallic catalysts and the application of in-situ spectroscopic techniques to liquid phase heterogeneously catalyzed reactions.

Achievements

The major activities of the PIRE program involve collaborative research among the PIRE partners and EU collaborators, research visits by students and faculty from the PIRE partner institutions to our EU collaborators, our annual PIRE meeting and an annual PIRE summer school. Each of these is described in more detail below.

PIRE Summer School, June 2011, Rolduc Abbey, Netherlands

The theme for last year's summer school was Energy and Materials from the Sun. The school was organized jointly with the European Graduate School on Sustainable Energy, a joint program of the Eindhoven University of Technology, the Technical University of Denmark and the Technical University of Munich. The PIRE program co-sponsored the event along with the South African consortium C*Change and other funding agencies in the Netherlands. A four day program was organized and attended by students from the US, EU and countries around the world. Most of the speakers were invited (with 31 invited lectures), but some students and post-docs also had an opportunity to speak at the meeting. There were more than 80 posters and a poster competition, with the winners including two PIRE students Carrie Farberow (UW) and Angelica Sanchez (UNM). A highlight of the meeting was the soccer match between Europe and the rest of the World, with a surprising win by the rest of the world. PIRE students actively participated in this event as well. A total of 14 students from the US attended the summer school (including one undergraduate) and 3 PIRE faculty (Shanks, Davis and Datye) and four invited speakers from other institutions in the US. Among the highlights of the educational program, a plenary session at the end of the day where all the speakers of the day were present to field questions from the audience. The central theme of the summer school was that solutions to many of the energy and environmental problems are tied to catalysts. Topics included photocatalysis, Fischer-Tropsch synthesis, chemical storage of solar energy and solar cells. The lectures included theory as well as industrial practice. The website http://www.europeansummerschool.eu provides further details on last year's summer school.

Annual PIRE Meeting, May 2011, Detroit, MI

The 2011 annual meeting was held on Sunday morning before the start of the North American Catalysis Society meeting in Detroit, MI. Having the meeting in conjunction with a national meeting made it easier for our collaborators from the EU to attend. We had four participants from the Netherlands (Bert Weckhysen and Krijn de Jong from Utrecht, Hans Niemantsverdreit from Eindhoven and K. Seshan from University of Twente. The meeting was very effective in getting an update on each other's research activities and formulating plans for future research.

Faculty and Student Research Visits 2011

During 2011, we had the following student doing research internships at our partner sites in the EU – Angelica Sancez (UNM) went to DTU and worked in the group of Ib Chorkendorff and with Thomas Hansen. Ryan Snell (ISU) spent the summer at the Fritz Haber Institute in Berlin working with Malte Behrens. David Hibbits (UVA) worked in the group of Bert Weckhuysen at Utrecht. And Tyne Johns (UNM) and Jonathan Paiz (UNM) worked with Peter Thune at Eindhoven. In addition, undergraduate students Elliot Coombs (ISU) and Valerie Ashbacher (UNM) worked at FHI in Berlin and Eindhoven respectively. These research visits allowed the students to become members of their host research groups over their stay and learn new experimental and theoretical

approaches. The research visits will lead to joint publications and the research will be included in the student dissertations.

During the year, we also had reciprocal visits from our EU collaborators, Prof. Peter Thune from Eindhoven spent two months at New Mexico to further develop our collaborations on model catalysts. During the stay he also gave some lectures on XPS, his field of expertise, and interacted with students in the UNM group. He now serves on the dissertation committee of Jonathan Paiz, and as a mentor to the PhD dissertation of Tyne Johns.

PIRE faculty member Robert Davis spent about three months in the Netherlands during the summer of 2011 as a visitor to the laboratories of Kriijn de Jong and Bert Weckhuysen at Utrecht University. While there, Davis participated in the PIRE summer school (Rolduc Abbey, June 19-23, 2011), presented invited seminars at Utrecht University (The Netherlands), Ecole Normale Supérieure de Lyon together with Institute for Research on Catalysis and the Environment (Lyon, France), Eindhoven University (The Netherlands), and the Dow Chemical Company (Terneuzen, The Netherlands). While in residence at Utrecht University, Davis met with many graduate students and postdoctoral researchers to discuss their research projects and potential collaborations with the PIRE team.

Research Highlights

The collaborative research with the team at Haldor Topsoe A/S has led to unique insights into the mechanisms of catalyst sintering. In-situ TEM of Ni/$MgAl_2O_4$ catalysts was performed at elevated temperatures and in the presence of H_2 and H_2O. The particle size of Ni particles was recorded as a function of time and by fitting a model to this data we were able to derive the energetic parameters that help explain the emission of atoms from these nanoparticles. The energetic parameters were used to predict the collective evolution of particle size in the entire catalyst. This is a unique approach to study catalyst sintering (Challa et al., JACS, 133(51): p. 20672-20675). The conventional approach is to perform studies at elevated temperatures over long times to gain an understanding of the stability of the catalyst. Our method relies on obtaining fundamental information on the ripening process obtained at very short times via in-situ TEM. Further work is needed to develop this method to arrive at robust models for understanding the long term stability of heterogeneous catalysts.

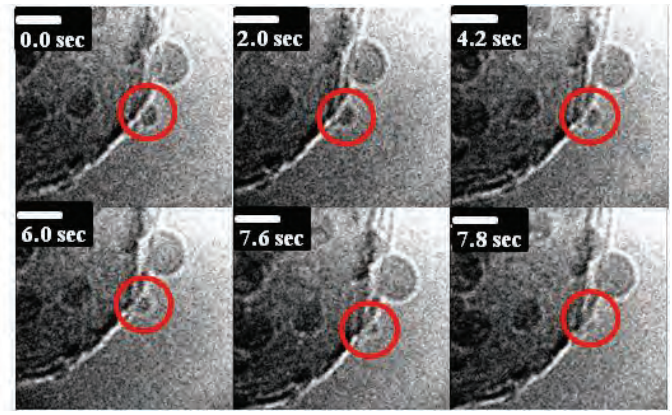


Figure 1. Time-lapsed TEM images of $MgAl_2O_4$-supported Ni nanoparticles at 750°C in 1:1 H_2:H_2O at a total pressure of 3.6 mbar. The scale bar in the images is 5 nm and the times are indicated relative to the start of observation (which is within a few minutes of reaching the operating temperature).

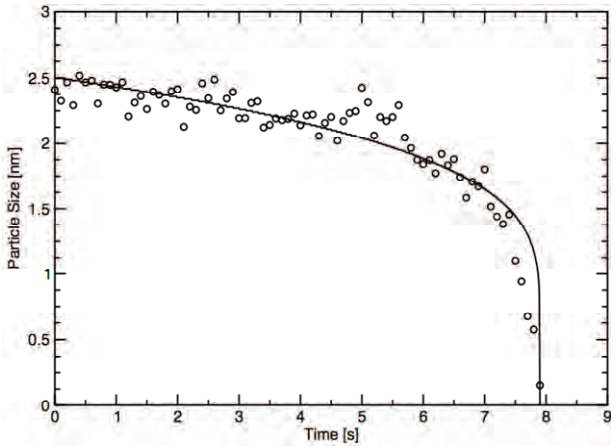


Figure 2. Size evolution of the Ni nanoparticle in Fig.1, experimental (circles) and best-fit predictions based on the equation below:

$$\frac{dr}{dt} = -\frac{K_{\text{int}}}{r}\exp\left(\frac{2\gamma\Omega}{rk_bT}\right),$$

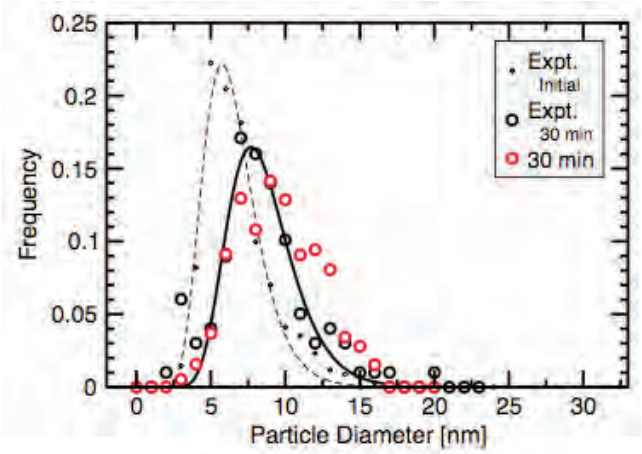


Figure 3. Size distributions before and after ripening for 30 minutes: experimental (black dots [initial] and circles [after 30 min]), and as predicted by our model $\frac{dr}{dt} = \frac{K_{\text{int}}}{r}\left[\exp\left(\frac{2\gamma\Omega}{r^* k_b T}\right) - \exp\left(\frac{2\gamma\Omega}{rk_bT}\right)\right]$. (red circles). The smooth curves are fits to the experimental data (broken-line [initial] and solid-line [after 30 min]) using a log-normal distribution.

Other Relevant Work

The PIRE partnership is unique in the field of catalysis since there are no large scale collaborations, to the best of our knowledge, between US and EU scientists.

Plans for the Next Year

During the coming year, we plan to complete publication of the book that will compile the lectures from the 2010 summer school. This was delayed due to the difficulties of publishing in an open access format, which was required by or collaborators from the Max Planck Society in Berlin. Hence, the publication is being handled by the Max Planck Society and will include open access in electronic form and also a print on demand through a commercial source to make this volume widely accessible.

Expected Milestones and Deliverables

We already have 18 collaborative publications resulting from the PIRE project and we expect many more in the coming year.

Member Company Benefits

Member companies get early access to the research done by our EU partner institutions.

Associated Project Abstracts

Provided in this section are abstracts for associated projects that are considered by CBiRC faculty to be integral to the center's research strategic plan or education strategic plan. In some cases, projects may have actually been awarded to non-ERC personnel, i.e., faculty and/or investigators outside the center, but partial funding was allocated directly to CBiRC faculty. To the extent practicable, current and proposed award year budget amounts for these projects as shown in Table 2, Volume I, reflect only the portion of such awards that is administered by the CBiRC faculty member's home department.

Further, in an effort to acknowledge other contributors/collaborators, CBiRC faculty members may have listed in their abstracts the names of the non-ERC PI/PD as Project Leader and non-ERC students and postdocs as team members. However, since these individuals were not directly involved in executing research funded by the center, or in carrying out ERC outreach activities, their demographic data were not collected, nor were they reported in Table 7 (ERC Personnel).

Thrust 1 – New Biocatalysts for Pathway Engineering

A Genetically Tractable Microalgal Platform for Advanced Biofuel Production

Sponsor:	U.S. Department of Energy
Project Leader:	Martin Spalding, Iowa State University
Other Faculty:	Basil J. Nikolau, David J. Oliver, and Eve Syrkin Wurtele, Iowa State University; John Morgan, Purdue University
Graduate Students:	Jason Hart, Iowa State University
Other Personnel:	Marcia Almeida-De-Macedo and Nick Ransom, Iowa State University

This research integrates innovative technical advances t develop a versatile, genetically tractable, microalgal-based platform to capture solar energy for the conversion of CO_2 to high-energy chemical products that have biofuel applications. The use of the highly tractable organism *Chlamydomonas* to hyper-accumulate reduced carbon products, such as oils, enables the iterative application of biotechnological- and genetic-based manipulations to optimize bioenergy production. Metabolic engineering of *Chlamydomonas* will be guided by state-of-the-art metabolite profile analyses, transcriptome sequence analyses and novel metabolic flux analyses. This project will generate new biofuel production capability, adaptable to a wide range of conditions and end products and with the transformational capability of genetically combining (i.e., breeding) a wide variety of desirable traits. This research will implement three primary technical objectives to enable industrial-level cultivation of *Chlamydomonas* for production of advanced biofuels: 1) optimize the metabolic partitioning of carbon to hyper-produce lipids by combining genetic engineering of candidate genes known to influence lipid biosynthesis with genetic screens for lipid hyper-accumulating mutants; 2) metabolically engineer an enhanced capacity for carbon assimilation; and 3) increase thermal tolerance to enable scale-up production.

** This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics

Sponsor:	National Institutes of Health
Project Leader:	Eve Syrkin Wurtele, Iowa State University
Other Faculty:	Basil J. Nikolau, Iowa State University
Other Personnel:	Marcia Almeida-De-Macedo, Nick Ransom and Ludmila Rizshsky, Iowa State University

Medicinal plants produce a wealth of pharmaceutical compounds such as taxol, vincristine, and morphine. Unfortunately, the specialized secondary metabolic pathways leading to such compounds remain poorly understood and progress in elucidating and manipulating these taxonomically restricted metabolic pathways has been correspondingly slow. This has been exacerbated by the limited development of "omics"-level resources for medicinal plants, which has meant that as a group, research in medicinal species have not benefitted to the same extent from the genomics revolution, as have research in model plants and agronomic crop species. This project combines the use of state-of-the-art sequencing technologies, metabolomics capabilities, and bioinformatics to develop an unrestricted, public resource to address this growing gap in our knowledge base of species-specific plant metabolism and accelerate the identification and functional analysis of genes involved in natural product biosynthesis in 20 widely used medicinal plant species. This resource will provide the research community with user-friendly access to the DNA sequences and expression profiles of each plant's transcriptome and associated metabolome, which we anticipate will have a t ranslational effect on understanding specialized metabolism, providing access to novel biocatalysts. To achieve this goal, we will utilize next generation sequencing approaches to determine the near-complete set of mRNAs encoded by each medicinal plant species. Transcriptome profiling of up t o 20 chemically diverse tissues/treatments per species using the RNA-Seq method from Illumina will be performed and correlated with metabolite profiles generated through LC-TOF and GC-MS for these same samples. All sequence and gene expression data will be deposited into NCBI and made available, along with metabolite profiling data at medicinalplantgenomics.msu.edu, a custom website developed by the research consortium. Thus, this project will provide searchable and downloadable databases for plant gene sequences, expression profiles and metabolites that can be accessed and utilized by the research community to facilitate discovery of the pathways and genes responsible for biosynthesis of key metabolites. High-throughput sequencing of genomes and transcriptomes has revolutionized and accelerated the pace and progress of research across the life sciences, and this project will for the first time extend these advances into the medicinal plant arena on a broad scale.

* *This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Annotation of Novel Enzymatic Functions in Methanogens

Sponsor:	U.S. Department of Energy
Project Leader:	Basil J. Nikolau, Iowa State University
Graduate Students:	Lucas Showman, Iowa State University
Other Personnel:	Libuse Brachova, Iowa State University

This project is developing an integrated high-throughput approach to functionally annotate a large group of conserved hypothetical genes in the methanogenic Archaea, *Methanosarcina acetivorans*. The focus is on genes predicted to encode enzymes (novel biocatalysts), the substrate(s) and products of which are unknown. Approximately 2,226 of the 4,524 genes in *M. acetivorans* fall into this category and include genes possibly involved in processes such as methanogenesis, nitrogen fixation, and carbon metabolism. The biochemical functions of these putative enzymes will be accurately annotated using a combination of gene knockouts, high throughput metabolomic analysis with mass spectrometry (MS), automated screening of implicated metabolites with nuclear magnetic resonance spectroscopy (NMR), and biochemical assays.

** This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Biocatalysts of the Acetyl-CoA Condensation Metabolic Pathway

Sponsor:	Iowa State University
Project Leader:	Basil J. Nikolau, Iowa State University
Postdocs:	Huanan Jin, Iowa State University

This project investigates how acetyl-CoA is metabolized via condensation mechanisms. The biocatalytic condensation of two acetyl-CoA molecules to form acetoacetyl-CoA is the initial reaction in two distinct metabolic pathways. In higher plants this is the initial reaction of the mevalonate pathway of isoprenoid biosynthesis in the cytosol of plant cells, and in photosynthetic microbes, it's the initial reaction in the biosynthesis of polyhydroxyalkanoates. These biocatalysts share common chemical mechanisms with the fatty acid synthase/polyketide synthase set of pathways. This project seeks to explore the suitability of these biocatalysts in the platform that CBiRC is establishing for the generation of 4-6 carbon platform chemicals for biorenewable applications. To date, these enzymes have been isolated and expressed in a recombinant system, and each biocatalyst is being characterized to ascertain detailed metabolic and structural understanding.

** This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Biosynthesis of Alkamides – Experimental Modeling of a Modular Secondary Metabolic Pathway

Sponsor:	National Science Foundation
Project Leader:	Basil J. Nikolau, Iowa State University
Other Faculty:	Robert Minto, Indiana University – Purdue University at Indianapolis
Postdocs:	Huanan Jin, Iowa State University
Other Personnel:	Ludmila Rizshsky, Iowa State University

This project is testing the feasibility of strategically applying high-throughput global profiling technologies to assess the expression of a complex genome and elucidate natural product biosynthetic pathways in a non-model species with an uncharacterized genome. Deciphering and defining the metabolic capability of the *Echinacea* genus to biosynthesize alkamides will test this

strategy. Alkamides are a class of specialized metabolites that are biologically assembled via a modular metabolic pathway that may be an adaptation of amino acid and fatty acid metabolism. Expedient and informative experimental systems have been proposed that will combine metabolite profiling and metabolic flux studies, coupled with the transcriptomics analysis of alkamide biosynthetic tissues to identify genes and enzymes that assemble a diverse collage of alkamides. Specifically, studies of the alkamide pathway therefore offer the potential of discovering new metabolic processes and associated biocatalysts that generate novel combinations of chemical functionalities (fatty amides, alkyl chains with carbon-carbon double and triple bonds arranged with unusual regiochemistry), which have wide-ranging applications (e.g., lubrication and detergent industries). In addition, this project outlines a general methodology that should be broadly applicable to discovering how primary and specialized plant metabolism is juxtaposed and evolves to generate the physiochemical phenotypic differences among plant taxonomic groups. The proposed multilayered bio-prospecting offers the opportunity to browse the metabolic repertoire of an organism and, with system-wide knowledge of the involved biochemical processes, should translate to the creation of novel bio-derived compounds relevant to the chemical industries, as well as strategies for pest- or disease resistance.

* *This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Coenyzme B12-dependent 1,2-propanediol degradation in *Salmonella*

Sponsor:	National Science Foundation
Project Leader:	Thomas A. Bobik, Iowa State University
Undergraduate Students:	Stephanie Cline, Iowa State University
Graduate Students:	David Gogerty, Iowa State University
Postdocs:	Fan Chenguang, Sharistha Sinha, and Shouqiang Cheng, Iowa State University
Other Personnel:	Yea Won Sung, Iowa State University

The long-term goal of this research is to determine the specific functions of the 23 genes involved in 1,2-PD degradation, and to elucidate the molecular principles of the microcompartments involved in this process. The specific aims focus on the structure, function and assembly of the microcompartments involved in 1,2-PD degradation, and on B12 recycling. Microcompartments provide a controlled environment for optimization enzyme catalyzed reactions. An understanding of their functional and structural principles will provide information helpful for engineering designer microcompartments to enhance the production of renewable chemicals.

Functional Genomics of the Biotin Metabolic Network of *Arabidopsis*

Sponsor:	Iowa State University
Project Leader:	Basil J. Nikolau, Iowa State University
Other Faculty:	Eve Syrkin Wurtele, Iowa State University
Other Personnel:	Libuse Brachova, Iowa State University

This project investigates how the essential vitamin, biotin, whose primary function has been ascribed as a catalytic cofactor required by enzymes involved in diverse metabolic processes, regulates a number of distinct metabolic functions. This work is based on prior studies conducted by the PI's (Nikolau and Wurtele) indicating that biotin is a regulatory molecule that appears to play critical roles in controlling transcriptional and post-transcriptional mechanisms in gene expression. This project seeks to identify and characterize the biochemical and physiological functions of genes associated with the biotin metabolic network of eukaryotic organisms, using *Arabidopsis* as a model. Plants are ideally suited for these studies as they, along with some microbes, are the primary organisms that can synthesize this molecule de novo; all other organisms must acquire this molecule from their diets or from the environment. The biotin network is defined as encompassing the genes that are involved in the biosynthesis, utilization, recovery and transport of biotin, genes coding for biotinylated protein, genes that are involved in a biotin-requiring process, and genes whose expression is altered by the biotin status of the organism. This project combines reverse genetic, biochemical, and molecular approaches to elucidate the functions of each of the genes associated with the biotin network. Characterization of these genes is occurring by the recombinant expression of each gene in a transgenic microbe, followed by biochemical characterization. To date, these characterizations have set the stage for the recombinant reconstitution of the following enzymes/pathways: 1) the plant biotin biosynthetic pathway that is constituted by 4 catalytic functions; 2) the biotin-dependent heteromeric acetyl-CoA carboxylase, consisting of 4 distinct catalytic components; and 3) a biotin-transporter function that catalyzes the movement of biotin between cells.

* *This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Mechanistic and Structural Basis for Plant Metabolic Evolution

Sponsor:	Howard Hughes Medical Institute
Project Leader:	Joseph P. Noel, Salk Institute for Biological Studies
Graduate Students:	Nikki Dellas and Chris Vickery, Salk Institute for Biological Studies
Postdocs:	Jing-Ke Weng, Ryan Philippe, and Hyun Jo Koo, Salk Institute for Biological Studies
Other Personnel:	Gordon Louie and June Brennan, Salk Institute for Biological Studies

What shapes natural selection of specialized enzymes and metabolic pathways underlying the emergence and expansion of chemical diversity in living systems remains a fundamental yet largely unanswered question in evolutionary biology. For sessile organisms possessing the developmental and ecological complexity of plants, this adaptive process is especially critical to their survival. The chemical output of these metabolic pathways serve as key mediators of intra- and interspecies interactions resulting in speciation, survival and ecological homeostasis. Specifically, we probe the adaptive molecular changes that have occurred in plant specialized metabolism as these enzyme networks emerged and subsequently evolved from their ancestral roots in primary metabolism at the dawn of terrestrial plants. Our work examines the biosynthesis of plant natural products including isoprenoids, phenylpropanoids, polyketides and associated flavonoids and fatty acid-derived metabolites. Specialized metabolic pathways and their "chemical output" present us with a rich evolutionary record of where biosynthetic

pathways, natural chemicals and biosynthetic enzymes have been (vestigial biochemical traits), what adaptive advantages these complex enzymatic systems hold in the present (emergent function), and ultimately where these pathways may be heading in the future (functional plasticity). Our decade-long study of these metabolic pathways has coalesced over the ensuing five years to answer a series of fundamental questions regarding the origin of specialized metabolism during land plant evolution. (i) Can one discern the phylogenetic routes through which plant secondary metabolic enzymes evolved from their primary metabolic ancestors? (ii) What are the biophysical features inherited by these enzymes that give rise to evolvability and/or restrain such evolutionary processes? (iii) How was the evolutionary directionality maintained if at all before the emergence of the ultimate activities that provide obvious selective advantages? (iv) What role did catalytic promiscuity play in shaping the evolvability of these biosynthetic systems? Answering these questions not only will extend our understanding of the biochemical strategies that early land plants adopted in their adaptation to a myriad of terrestrial environments, but will also better shape our appreciation of mutability and the origins of new enzyme function in general.

Mechanistic, Structural and Evolutionary Basis for Phenylpropanoid Metabolism

Sponsor:	National Science Foundation
Project Leader:	Joseph P. Noel, Salk Institute for Biological Studies
Undergraduate Students:	Justin Pacheco, Salk Institute for Biological Studies
Graduate Students:	Helena Sun, Salk Institute for Biological Studies
Postdocs:	Jing-Ke Weng, Charisse Crenshaw, Ryan Philippe, and Charles Stewart, Jr., Salk Institute for Biological Studies
Other Personnel:	Gordon Louie and Marianne Bowman, Salk Institute for Biological Studies

The phenylpropanoid pathway of bacteria and plants provides a system to decipher the core principles influencing evolutionary change in enzymes and metabolic pathways underlying the emergence and rapid expansion of chemical diversity in living systems. Ultimately these studies lead to a better understanding of the chemical, structural and evolutionary tenets governing biodiversity and biocomplexity at a chemical level. Sessile organisms such as plants and microbes acquired and evolved specialized biosynthetic networks classified as secondary metabolic pathways, the output of which are regio- and stereo-chemically complex small molecule natural products including phenylpropanoid-derived metabolites. These chemicals of specialized metabolism serve as chemical languages in ecosystems and impart a species-specific chemical "signature" on the parent organism. The means by which organisms acquire, improve and exploit diverse metabolic systems to generate a rich repertoire of chemically complex natural products play key roles in the rapid expansion of many ecosystems, and therefore, hold incredible adaptive significance for the diversity of life. While seemingly insignificant, specialized metabolites often serve as key mediators of intra- and interspecies interactions resulting in speciation, survival and ecological homeostasis. Under the evolutionary restraints of chemically established adaptation, diverse molecular changes associated with specialized metabolism are often preserved genetically in a particular species' genome and are discerned at a functional and structural level. These often ecotype specific genomes are the direct result of the increased fitness of host organisms "chemically" adapted to specific ecological niches.

Therefore, these specialized metabolic pathways and their "chemical output" present us with a rich evolutionary record of where biosynthetic pathways, natural chemicals and biosynthetic enzymes have been (vestigial biochemical traits), what adaptive advantages these complex enzymatic systems hold in the present (emergent function), and ultimately where these pathways may be heading in the future (functional plasticity). The overarching goal of this project is to map the adaptive molecular changes that have occurred in the phenylpropanoid biosynthetic pathway as these enzyme networks emerged and subsequently evolved from their ancestral roots in primary metabolism billions of years ago. To accomplish these goals, the work involves a multidisciplinary approach including synthetic chemistry, protein x-ray crystallography, site-specific and combinatorial mutagenesis, kinetic assays and research using the reference plant *Arabidopsis thaliana* to answer unresolved, recently discovered and unexpected evolutionary aspects of the general phenylpropanoid biosynthetic pathway.

Metabolomics: A Functional Genomics Tool for Deciphering Functions of *Arabidopsis* Genes in the Context of Metabolic and Regulatory Networks

Sponsor:	National Science Foundation
Project Leader:	Basil J. Nikolau, Iowa State University
Other Faculty:	Julie Dickerson, Philip Dixon, and Eve Syrkin Wurtele, Iowa State University; Ruth Welti, Kansas State University; Lloyd Sumner, Noble Foundation; Sueng Rhee, Stanford University; Oliver Fiehn, University of California – Davis
Graduate Students:	Jennifer Robinson and Xin Guan, Iowa State University
Other Personnel:	Stephanie Moon, Iowa State University

Global profiling technologies enable comprehensive overview of the consequences of genetic alterations and can be used to annotate gene functions. However, the functions of over one-third of the annotated protein-coding genes of the *Arabidopsis* genome are still unknown, and the annotation of an even larger portion of the genome is not sufficiently accurate for unambiguous assignment function at the biochemical and physiological levels. This project builds on a prior pilot project that enabled a consortium of multidisciplinary collaborators to establish pipelines for generating metabolomics data streams and to integrate the outcomes with bioinformatics, computational, and database capabilities. Our goal is to develop novel capabilities that will enhance the research community's ability to formulate testable hypotheses concerning *Arabidopsis* gene function. The consortium has developed metabolomic platforms that together detect approximately 1,800 metabolites, of which 900 are chemically defined. The aims of the current project is to apply these established platforms to reveal changes in the metabolome associated with knockout mutations in 450 genes of unknown function and compare these to similar mutants in 50 genes of known function. To enhance the power of the metabolomics platforms, the consortium will begin analytical efforts to expand the chemical identity of the *Arabidopsis* metabolome. Finally, the consortium will disseminate these data via the multi-functional metabolomics database developed in the pilot project. Enhancement of this database and associated statistical and visualization toolsets will enable researchers to formulate testable computational models of the metabolic network of *Arabidopsis*. The successful completion of these goals and integration with other NSF-sponsored functional genomics and cyber

infrastructure developments will generate transformational resources for ultimately modeling the complex metabolism of *Arabidopsis*.

** This project is relevant to, and integrates across, both Thrust 1 and University Education/ Outreach.*

Uncovering Novel Signaling Interactions in Plant Metabolic Networks

Sponsor:	National Science Foundation
Project Leader:	Eve Syrkin Wurtele, Iowa State University
Other Faculty:	Ling Li, Iowa State University
Undergraduate Students:	Marah Hoel, Alan Kading, and Robert Goetz, Iowa State University
Graduate Students:	Micheline Ngaki, Iowa State University

This project investigates proteins of unknown function that regulate metabolism in plants. The work is important in that relatively little is known about the factors that influence carbon partitioning in cells, yet this understanding is crucial to achievement of high yields of desired compounds. The genes we identify in this study might function directly in yeast, or have a homolog in this organism. F urthermore, the mechanisms of metabolic regulation may be common across multiple organisms.

** This project is relevant to, and integrates across, both Thrust 1 and University Education/ Outreach.*

Thrust 2 – Microbial Metabolic Engineering

A Native Pathway for the Production of n-Butanol in *Escherichia coli*: A New Paradigm for Synthetic Biology

Sponsor:	National Science Foundation
Project Leader:	Ramon Gonzalez, W. M. Rice University
Graduate Students:	S. Young and S. Dash, W. M. Rice University
Postdocs:	James Clomburg, Jake Vick, W. M. Rice University

Interest in the use of advanced biofuels, such as n-butanol and other higher-chain linear alcohols, has rapidly developed because they offer several advantages compared to ethanol, including less hygroscopicity and volatility, higher energy density and compatibility with current infrastructure for storage, distribution and usage. Among linear alcohols currently considered as advanced biofuels, n-butanol is the only one found in nature as a major fermentation product. The ability to synthesize n-butanol is considered to be an exclusive feature of clostridial species. *Clostridia* are spore formers, obligate anaerobes that grow at slow rates, have complex nutritional requirements and produce n-butanol along with a mixture of other products including acetone, ethanol, butyrate, and acetate. The lack of efficient genetic tools to manipulate clostridia, along with their complex metabolism, hinders metabolic engineering efforts that could lead to the improvement of n-butanol yield, titer, and productivity. In an effort to overcome the aforementioned issues, the genes that enable the synthesis of n-butanol in native producers like

clostridia have been imported into industrial organisms that are genetically and metabolically tractable such as *E. coli*, *Saccharomyces cerevisiae*, *Pseudomonas putida*, *Bacillus subtilis*, *Lactococcus lactis*, and *Lactobacillus* species. All efforts to date have been based on what we refer to in this project as heterologous metabolic engineering (HeME): that is, transplanting genes/pathways of (primarily) clostridial origin to hosts otherwise not able to produce butanol (e.g. *E. coli*, *S. cerevisiae*). HeME-based approaches have been used to engineer biofuel production in the past and are currently viewed as the strategy of choice when the host organism does not possess the desired metabolic function. However, in the case of n-butanol and other linear n-alcohols, HeME approaches have faced significant hurdles. For example, after several years of strain development and optimization, organisms engineered for the production of n-butanol synthesize this alcohol at low flux and still require the supplementation of the medium with rich nutrients. We hypothesize that the use of a heterologous metabolic engineering approach represents the main issue accounting for the limited success of the aforementioned studies, as it relies on transferring a heterologous pathway that might not be compatible with the host, thus compromising its functionality.

The intellectual merit of the work relates to addressing the aforementioned limitations by developing an alternative strategy that focuses on the identification and harnessing of native *E. coli* enzymes/pathways that could act as surrogates of the heterologous n-butanol-synthesis pathway and hence mediate the synthesis of a non-native product in the absence of foreign genes. Since no exogenous gene is recruited to establish the otherwise foreign pathway, we have termed this approach "homologous metabolic engineering" (HoME). The overall goal of this project is to identify, characterize and harness native biosynthetic pathways for the efficient production of n-butanol in *E. coli*, thus establishing a new paradigm for the application of synthetic biology to the production of advanced biofuels. The specific objectives of the work are: i) identify native *E. coli* genes encoding enzymes that can catalyze the reaction steps comprising the clostridial butanol pathway; ii) in vivo assembly and functional characterization of a native butanol pathway in *E. coli*; iii) improve the efficiency of the native n-butanol pathway; and iv) system-wide characterization of wild-type and engineered strains.

The broader impacts of the project are numerous. The establishment of HoME as a new paradigm for metabolic engineering and synthetic biology would lead to exploiting the multi-potent capabilities of native hosts via engineering of functional differentiation. By enabling the production of n-butanol through a homologous pathway, this proposal will contribute to the creation of fundamentally new approaches that could enable efficient production of second-generation biofuels in many industrial organisms. Based on these advances, efficient and economically viable chemical and biofuel industries can be developed that will make possible energy independence and climate protection. This project will also educate our society in the scientific and engineering challenges and opportunities on the road to a sustainable energy future. We will capitalize on our collaborations with the Houston Harmony Science Academy to train middle and high schools students in the field of alternative energy. This school serves predominantly minority populations, and thus, we will address the national need, and challenge, of increasing their participation in science and engineering.

A Robust Platform for Reconstituting and Engineering Iterative Megasynthases

Sponsor:	National Institutes of Health
Project Leader:	Yi Tang, University of California, Los Angeles
Other Faculty:	Nancy A. Da Silva, University of California – Irvine
Undergraduate Students:	Marc Kryger, University of California – Irvine
Graduate Students:	Jin Wook Choi, University of California – Irvine

Nature uses an amazing array of enzymes to make small molecule natural products. Among the most interesting but least understood enzymes making these compounds are the iterative polyketide synthases (IPKSs) found in filamentous fungi. In contrast to the well-studied bacterial type I PKSs that operate in an assembly-line fashion, IPKSs are megasynthases that function iteratively by using a single set of catalytic domains repeatedly in different combinations to produce structurally diverse fungal metabolites. Bioinformatics analysis of the genomes of recently sequenced fungal species revealed that each genome contains a l arge number of genes encoding IPKSs. The total numbers of IPKSs significantly outnumber the known polyketides and polyketide-nonribosomal peptides isolated from these species, suggesting that a majority of biosynthetic genes are silent in these fungi under cultivating conditions. This in turn suggests that the fungal species may have untapped potential to synthesize a much larger number of natural products. Furthermore, analysis and engineering of IPKSs have been hampered by inability to obtain sufficient amounts of the functional purified megasynthase from either the native fungal host or heterologous *Aspergillus* hosts. As a result, the programming that governs metabolite assembly by IPKSs is not understood. Key aspects that remain to be elucidated include: 1) the catalytic and structural roles of each domain in the megasynthase 2) substrate specificities of the catalytic domains and their tolerance to perturbation in megasynthase functions; and 3) actors governing the choice of different combinations of catalytic domains during each iteration of catalysis. The objective of this project is to develop the genetically superior *Saccharomyces cerevisiae* as a h eterologous host for reconstitution, analysis and engineering of IPKSs, especially the enigmatic highly-reducing IPKS, such as LovB associated with Lovastatin biosynthesis. We have accumulated a significant body of preliminary data to demonstrate that *S. cerevisiae* is a highly robust host for expressing these megasynthases in functional forms, and can facilitate the production of polyketide products both in vivo and in vitro with purified enzymes. The following specific aims will be pursued: 1) Engineer and optimize *S. cerevisiae* towards producing fungal metabolites and megasynthases; 2) reconstitution of fungal megasynthases in *S. cerevisiae*; 3) biochemical analysis of fungal PKS using *S. cerevisiae*; and 4) genome mining of filamentous fungi using *S. cerevisiae* as a host.

* *This project is relevant to, and integrates across, both Thrusts 1 and 2.*

CAREER: Understanding and Harnessing the Fermentative Metabolism of Glycerol in *E. coli* – A New Path to Biofuels and Biochemicals

Sponsor:	National Science Foundation
Project Leader:	Ramon Gonzalez, W. M. Rice University
Graduate Students:	James Clomburg and Angela Cintolesi W. M. Rice University

Although biofuels such as biodiesel and bioethanol represent a sustainable, secure, renewable, and environmentally safe alternative to fossil fuels, major scientific and technological breakthroughs are needed for them to become economically viable. The long-term goals of this research are to elucidate the pathways and mechanisms mediating the anaerobic fermentation of glycerol in *E. coli* and use the knowledge base thus created to engineer this organism for the efficient production of reduced chemicals and fuels. The specific objectives of the research plan are: (1) Study the effect of medium composition and cultivation conditions on glycerol fermentation, (2) assess the role of hypothesized pathways in the fermentative metabolism of glycerol by using genetic and biochemical approaches; (3) identify genes, proteins, and metabolic processes involved in the fermentation of glycerol using functional genomics tools; and (4) engineer *E. coli* for the efficient co-production of ethanol and hydrogen, thus illustrating the advantages of using glycerol fermentation as a new platform to produce biofuels and biochemicals. To achieve these goals, functional genomics tools will be used to supplement a traditional hypothesis-driven approach. In addition, metabolic engineering will be used as a rational approach to engineer *E. coli* for the conversion of glycerol into ethanol and hydrogen. The intellectual merit of this project relates to elucidating the fermentative metabolism of glycerol in *E. coli*, a puzzle that has remained unresolved for more than eighty years. By enabling and integrating the production of biofuels, this project will contribute to the creation of fundamentally new processes and paradigms such as those embracing the biorefinery concept. This research will also make significant contributions to the education of our society on the role of biofuels as enablers of a secure and sustainable energy future.

Collaborative Research: Metabolic Engineering of Terpenoid Indole Alkaloids Using Transcriptional Regulators in C. roseus Hairy Roots

Sponsor:	National Science Foundation
Project Leader:	Jacqueline V. Shanks, Iowa State University
Graduate Students:	Le Zhao

Plants produce large numbers of chemicals that are useful as drugs for treating a variety of medical conditions. Unfortunately, these chemicals are often produced by plants in only trace amounts. As a result, large quantities of plant material must often be used to produce small amounts of drugs, making these drugs very expensive. An example of this problem is provided by a class of compounds produced by *Catharanthus roseus* (periwinkle), known as terpenoid indole alkaloids. Terpenoid indole alkaloids include chemicals such as vincristine and vinblastine, which are used to treat certain types of cancer, and ajmalicine, which is used to treat hypertension. As periwinkle produces these chemicals in only trace amounts, they can cost over one million dollars per pound. To reduce the costs of these drugs, it is critical to develop varieties of periwinkle that produce these drugs in higher amounts. Towards that end, the effects of increasing expression of the biosynthetic genes that are responsible for production of these drugs will be investigated. A two-fold strategy for increasing expression of these biosynthetic genes will be pursued. Part one of this strategy is to test the effects of reducing expression of repressor genes that decrease expression of the biosynthetic genes. Part two of this strategy is to decrease expression of the repressor genes while simultaneously increasing expression of activator genes that increase expression of the biosynthetic genes. The hypothesis being tested is that, by decreasing expression of repressors and increasing expression of activators, it should be

possible to achieve a significant increase in expression of the biosynthetic genes. This increase in expression of the biosynthetic genes should then lead to increased production of terpenoid indole alkaloids, allowing those terpenoid indole alkaloids that are useful as drugs to be produced at a lower cost. Previously uncharacterized chemicals that accumulate in response to alterations in expression of the biosynthetic genes will also be analyzed. Characterization of these chemicals will provide valuable information regarding the biochemical pathways by which terpenoid indole alkaloids are produced, as well as potentially providing information regarding additional chemicals that may be tested for use as drugs.

Designed Feedback Regulation of Electron Transport Energetics

Sponsor:	National Institutes of Health
Project Leader:	Ka-Yiu San, W. M. Rice University
Other Faculty:	George N. Bennett

The focus of the project is to employ both novel genetic control circuits and extensive experimental analysis to position the redox energetics of the *E. coli* cell to the optimal physiological and biochemical state for production of chemicals operating in a microaerobic mode. We will engineer cells that have an optimized window of function of the electron transport system allowing suitable growth and cell energetics while allowing a high portion of the NADH to be used in the desired biocatalyst mode for product formation. For feedback control of individual proteins required for ETS function, we will express an essential gene under control of the Arc system such that it is only on as the cells become more aerobic, while at the same time expressing another gene essential for proper ETS function under a different control system so it exhibits a feedback response evoking a defined functional level of ETS in a prescribed manner as oxygen and culture conditions are changed. Once a series of constructs are made with altered ETS levels and response patterns, preliminary culture studies will be conducted to see which are most likely to be useful in practical applications.

Efficient Synthesis of Hydrocarbons Using an Engineered Reversal of the β-Oxidation Cycle: A New Paradigm for the Production of Advanced Biofuels

Sponsor:	National Science Foundation
Project Leader:	Ramon Gonzalez, W. M. Rice University
Graduate Students:	S. Kim, S. Dash
Postdocs:	James Clomburg, W. M. Rice University

In recent years much effort has been devoted to the production of biofuels, such as ethanol, butanol and higher-chain alcohols, via microbial fermentation of sugars and other biomass constituents. Interest in the use of advanced biofuels, such as hydrocarbons, has rapidly developed because they offer several advantages compared to alcohols, including less hygroscopicity and volatility, higher energy density and compatibility with current infrastructure for storage, distribution and usage.

Biosynthetic pathways leading to the synthesis of saturated (alkanes) and unsaturated (alkenes) hydrocarbons have been reported in the literature for decades, but not until recently were the corresponding biosynthetic genes identified. The “head-to-head” condensation of acyl-CoA

thioesters has been proposed as the primary pathway to generate long-chain alkenes. On the other hand, alkanes are synthesized through a two-step pathway that involves conversion of acyl-CoA thioesters to fatty aldehydes, which are then decarbonylated to alkanes. To date, the fatty acid biosynthesis pathway has been used as the exclusive means to generate the aforementioned acyl-CoA thioesters required for the synthesis of hydrocarbons. However, the operation of this pathway is not efficient because it consumes ATP in the synthesis of malonyl-ACP, which is the donor of two-carbon units for chain elongation. As a consequence, the ATP yield associated with the production of hydrocarbon through the fatty acid synthesis pathway is very low. This, in turn, greatly limits cell growth and hydrocarbon production.

In this study, we use a functional reversal of the β-oxidation cycle, which was recently engineered in our laboratory, as a metabolic platform for the synthesis of hydrocarbons. Unlike the fatty acid biosynthesis pathway, the reversal of the β-oxidation cycle operates with coenzyme-A (CoA) thioester intermediates and uses acetyl-CoA directly for acyl-chain elongation (rather than first requiring ATP-dependent activation to malonyl-CoA). These characteristics enable product synthesis at maximum carbon and energy efficiency. We have already demonstrated the superior nature of this metabolic platform by producing higher-chain linear n-alcohols ($C \geq 4$) and extracellular long-chain fatty acids ($C > 10$) at yields and titers an order of magnitude higher than previously reported.

The intellectual merit of this project relates to the efficient synthesis of hydrocarbons using an engineered reversal of the β-oxidation cycle, which in turn will establish a new paradigm for the production of advanced biofuels. Four specific objectives are proposed to accomplish this: i) Engineer a functional reversal of the β-oxidation cycle with a minimal set of enzymes; ii) engineer pathways for the synthesis of alkanes and alkenes (olefins) from acyl-CoA intermediates generated in the functional reversal of the β-oxidation cycle; iii) improve the efficiency of the engineered reversal of the β-oxidation cycle during the synthesis of hydrocarbons; and iv) system-wide characterization of wild-type and engineered strains.
The broader impacts of the work are numerous. The ubiquitous nature of β-oxidation enzymes should enable the combinatorial synthesis of non-native products in industrial organisms with a minimum number of foreign genes, an approach that would ensure the efficient functioning of the engineered pathways. By enabling the production of hydrocarbons through a functional reversal of the β-oxidation cycle, this proposal will contribute to the creation of fundamentally new approaches that could enable efficient production of second-generation biofuels. Based on these advances, efficient and economically viable chemical and biofuel industries can be developed that will make possible energy independence and climate protection. This proposal will also educate our society in the scientific and engineering challenges and opportunities on the road to a sustainable energy future. We will capitalize on our collaborations with the Houston Harmony Science Academy to train middle and high schools students in the field of alternative energy. This school serves predominantly minority populations, and thus we will address the national need, and challenge, of increasing their participation in science and engineering.

EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels

Sponsor:	National Science Foundation
Project Leader:	Jacqueline V. Shanks, Iowa State University
Other Faculty:	Thomas A. Bobik, Basil Nikolau, D. Raj Raman, Govind Nadathur, and Gordon Wolfe, Iowa State University
Postdocs:	Geng Ding and Guy Sander, Iowa State University
Graduate Students:	Mark Brown, Jennifer Chmielowski, Shivani Garg, Adarsh Jose, and Wenmin Qin, Iowa State University
Undergraduate and REU Students:	Emily Yun Lin, Kim-Xuan Dinh Nguyen, Udochukwu Okorafor, Samson Condon, Robyn Rourke, Julia Petrullo, Morgan Becker, and Miya Williams
Other Personnel:	Libuse Brachova, Ann Perera, Kathy Wiederin, Xiaobin Zheng, Jong Moon Yoon and Marna Yandeau-Nelson, Iowa State University

This project will develop new bio-engineering technology for transforming the current liquid fuel industry from using fossil-carbon feedstocks to using biorenewable feedstocks that are at the chemical level identical to gasoline and diesel fuels, namely biologically-generated hydrocarbons. The engineering system we envision is a photosynthetic-based organism that will have the bio-engineered ability to chemically-reduce atmospheric CO_2 to simple hydrocarbons (e.g., n-alkanes and n-alkenes), using sunlight as the source of renewable energy. Such metabolic conversions are known to occur in discreet places in the biosphere, e.g., the epidermis of plants and insects, and as a carbon/energy-storage mechanism by certain algae. Our goal is to conduct multidisciplinary studies that will identify the mechanisms and genetic elements that encode the biocatalyst(s) that generate these hydrocarbons in biological systems. We will explore the use of these isolated genetic elements to bio-engineer crops or bioengineer photosynthetic microbes as the production platform to realize the vision of producing a biological hydrocarbon based-fuel. The proposed research will for the first time lead to fundamental knowledge concerning the structure and mechanism of the biocatalyst that generates biological hydrocarbons. And, the efficient use of this novel biocatalyst in a production biological host will require the optimization of bioengineering principles so as to proficiently integrate the biocatalyst into a pre-existing metabolic network without compromising the biological competence of the host. These later optimizations will integrate concepts of biological control principles with engineering proficiencies. This project brings together a collaborative team of biologists and engineers to demonstrate a paradigm of how fundamental molecular biological research can be integrated with disciplines of engineering to generate new bio-engineered organisms that can be used as a sustainable production platform to meet the global demands for new liquid biofuels. An REU program and an international collaborative are venues for training undergraduate students and graduate students/postdoctoral associates, respectively.

** This project is relevant to, and integrates across, both Thrusts 1 and 2.*

Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator

Sponsor:	ConocoPhillips Company
Project Leader:	Dennis Vigil, Iowa State University
Other Faculty:	Jacqueline V. Shanks, Iowa State University
Postdocs:	Bo Kong, Iowa State University

Large-scale production of biofuel from microalgae requires not only the development of elite fuel-producing microorganisms, but it also requires novel process engineering approaches that (1) significantly accelerate the rate of photosynthesis and (2) provide energy-efficient methods for harvesting and separating algae and biofuel products. One of the most important factors for increasing the algal photosynthesis rate is high-frequency periodic exposure of cells to light and dark regions of the suspending fluid, and such high-frequency periodic exposure cannot be achieved in pond systems or conventional photobioreactors. Furthermore, since algae ponds and photobioreactors do not provide any natural mechanisms for harvesting mature algae and recovering biofuel products, expensive downstream separation processes must be employed. The purpose of this work is to develop a novel continuous-flow reactor-separator that exploits a highly efficient self-organized flow pattern (Taylor vortices) to increase the rate of photosynthesis by rapidly shuttling microorganisms between light and dark regions of the reactor while simultaneously achieving centrifugal product separation. The proposed reactor-separator offers the additional advantages of good contacting of gas and liquid phases, low probability of rupturing algal cells, and nearly ideal plug flow behavior. A preliminary analysis suggests that this novel algal reactor-separator has the potential to produce biofuel at an energy cost that is approximately 8% of the energy produced, compared to an estimated 30% for conventional photobioreactors, which do not achieve product separation. The development and analysis of the Taylor vortex algae reactor-separator will be accomplished by building a bench-scale reactor-separator prototype; carrying out an experimental program to determine how geometric and operating variables influence photosynthesis rate and reactor-separator operating costs; and developing computational models suitable for evaluating the scale-up potential of the device.

Engineering an Efficient Biocatalyst for Chiral Compound Production

Sponsor:	National Science Foundation
Project Leader:	Ka-Yiu San, W. M. Rice University
Other Faculty:	George N. Bennett

The goal of this project is to develop efficient engineered strains to increase the cofactor NADPH availability for the production of chemicals that require NADPH for their biosynthesis. The scope of the project includes the construction of new engineered strains and the characterization and testing of these stains for their capability to production biochemicals.

Engineering Yeast Consortia for Surface-display of Complex Cellulosome Structures: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol

Sponsor:	National Science Foundation and U.S. Department of Energy
Project Leader:	Wilfred Chen, University of Delaware
Other Faculty:	Rachel Chen, Georgia Tech.; Nancy A. Da Silva, University of California – Irvine
Undergraduate Students:	Andres Aguirre, University of California – Irvine
Graduate Students:	Sneha Srikrishnan, University of California – Irvine

According to the new Energy Policy Act, a billion gallons of renewable fuel must be produced by 2012 with most of that produced as biofuel using renewable biomass. In particular, bioethanol from renewable sources provides an attractive form of alternative energy. The primary obstacle impeding the more widespread production of energy from biomass is the absence of a low-cost technology for overcoming the recalcitrance of these materials. It has been shown that the overall cost can be significantly reduced using a one-step "consolidated" bioprocessing (CBP) of lignocellulose to bioethanol, where cellulase production, cellulose hydrolysis and sugar fermentation can be mediated by a single microorganism or microbial consortium. Cellulosomes are self-assembled multi-enzyme complexes presented on the anaerobes' cell surface and are dedicated to cellulose depolymerization. This self-assembled system brings multiple enzymes in close proximity to the substrate, and provides a structure that ensures high local concentration and the correct ratio and orders of the enzymes, thereby increasing cellulose hydrolysis synergy up to 50-fold. The objective of this project is to develop a synthetic yeast consortium for direct fermentation of cellulose to ethanol with productivity, yield, and final concentration close to that from glucose fermentation. The specific objectives are: 1) Construct a yeast consortium for surface assembly of a mini-cellulosome structure consisting of three cellulases and demonstrate the feasibility of using the consortium for direct ethanol production from cellulose; 2) construction of yeast strains for surface-display of the anchoring scaffoldin, strains for secreting the adaptor scaffoldin, and strains for secreting the dockerin-tagged cellulases; and 3) demonstrate the feasibility of the constructed yeast consortium to display the complex cellulosome and the ability for direct fermentation of cellulose to ethanol. The engineering strategy proposed emphasizes the efficiency of hydrolysis and synergy among cellulases, rather than focusing on the amount of enzymes produced or used.

Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed

Sponsor:	Pioneer Hi-Bred International, Inc.
Project Leader:	Jacqueline V. Shanks, Iowa State University
Undergraduate Students:	Ezinne Archinivu, Miya Williams and Kae Koch, Iowa State University
Graduate Students:	Quyen Truong, Iowa State University

The factors that control resource partitioning in soybeans are poorly understood but are of great economic importance due to a strong negative correlation between oil and protein contents. Here we propose to use soy somatic embryos as models for the analysis of resource partitioning using

metabolic flux analysis. Should the embryo system prove to be a suitable model (show reproducible changes in pathway fluxes in response to experimental perturbations), we propose to probe for flux control points in a series of experiments designed to influence assimilate partitioning using nutritional, physical and transgenic perturbations. Potential control points, identified during the initial phases of experimentation, will be targeted using transgenic techniques in subsequent experimentation.

Fermentative Utilization of Pyrolytic Substrates

Sponsor:	National Science Foundation
Project Leader:	Laura Jarboe, Iowa State University
Undergraduate Students:	Lee Yeo, Iowa State University
Postdocs:	Kumar Babu Kautharapu, Iowa State University

The overall goal of this work is to use microorganisms to produce biofuels from biomass. This project is distinctive in that we aim to produce sugar substrates for these microorganisms by fast pyrolysis of biomass, instead of hydrolysis or gasification. Economical analysis has shown that this is a viable and competitive method for producing biofuels. We will produce two model biofuels or biofuel feedstock, ethanol and lipids, by two model microorganisms, *Escherichia coli* and *Chlamydomonas reinhardtii*. Fermentation of the fast pyrolysis product, also known as bio-oil or thermolytic substrates, is limited by the fact that in addition to useful substrates, it also contains many inhibitory "contaminant" compounds, including furans and phenols. At Iowa State University, we have a pyrolysis method that enables the collection of bio-oil in distinct fractions. This fractionation changes the distribution of substrates and of these inhibitory contaminant compounds. This decreases but does not eliminate the problem of contaminant toxicity. Addressing this toxicity is the main focus of this project.

We aim to increase the tolerance of our two microorganisms to these inhibitory contaminants, so that the need for additional purification processing of the pyrolysis product is reduced. We will use metabolic evolution to increase the robustness of our microorganisms to the contaminants, largely because the bio-oil is complex and the mechanism of the inhibition is not known. The outcomes of this work include microorganisms that can utilize raw thermolytic substrates for production of biofuels and a roadmap for engineering other microorganisms for this ability.

Generation of Biofuels from Abundant Non-Digestible Oilseed Components

Sponsor:	National Science Foundation
Project Leader:	Ka-Yiu San, W. M. Rice University
Other Faculty:	George N. Bennett

The production of biofuels from renewable resources is currently an area national interest. In particular, butanol isomers have gained interest as an alternative fuel due to their superior fuel properties relative to ethanol. This research will use metabolic engineering approaches to enable the fermentation of the non-digestible, soluble carbohydrate fraction isolated from many common oilseeds such as soybean, cottonseed, and sunflower seed, to butanol. Many of these materials are byproducts of biodiesel or bioethanol production. For example, large amounts of galactose-rich soluble carbohydrates, constituting approximately 10% of oil seed weight,

represent a sustainable yet underutilized feedstock for biofuel. Towards this end, the hydrolysis of the galactose-rich oligosaccharides and subsequent fermentation to 2-butanol by engineered *E. coli* will be studied and optimized. Furthermore, natural and engineered strains of *Clostridia* may also be able make effective use of this feedstock. Genetic modules for engineered strains of *Clostridia* will be developed that enable the efficient uptake and metabolism of galactose-rich oligosaccharides and their fermentation to 1-butanol.

Mass Spectrometric Imaging of Plant Metabolites

Sponsor:	U.S. Department of Energy
Project Leader:	Basil J. Nikolau, Iowa State University
Other Faculty:	Mei Hong, Robert Houk, Young-Jin Lee, Nicola Pohl, and Edward Yeung, Iowa State University
Postdocs:	Zhihong Song, Iowa State University

This project is developing mass spectrometric imaging techniques to map metabolite distributions within tissues, and eventually among individual cells. Mass spectrometry not only allows positive identification of the many metabolites but can also reveal the substrates and precursors involved in each metabolic pathway. Such information will provide unprecedented details on the distribution of metabolites from cell to cell, cooperative and antagonistic effects among the metabolites, and environmental influences on metabolism. Such details will ultimately lead to a predictive understanding of the mechanisms that multi-cellular organisms use to regulate metabolic processes. In the current work, we are focusing on the lipids of *Arabidopsis*. By studying the diversity of the lipids, we hope to gain detailed insight into their biosynthesis as a function of genetics, tissue type, development, and environment. In analogy to matrix-assisted laser desorption ionization (MALDI), a laser beam will be used to interrogate sequentially micrometer areas of a plant by vaporizing the surface contents of the tissue into a mass spectrometer. Rastering of the laser beam over the tissue will produce a laterally resolved image of the various substances within different structures of the plant. Repeated vaporization at the same focused point of a plant structure will produce a depth profile of the components. We plan to generate ions directly from the plant tissue by designing novel additives as pseudomatrixes. By minimizing sample preparation, compositional integrity and spatial resolution of the analysis will be guaranteed. Identification of the metabolites will be aided by new strategies in carbohydrate sequencing and in 2D-NMR.

** This project is relevant to, and integrates across, both Thrusts 2 and 3.*

Metabolic Engineering of *Moritella marinus* to Produce DHA

Sponsor:	Metabolic Technologies, Inc.
Project Leader:	Laura Jarboe, Iowa State University
Undergraduate Students:	Lee Yeo, Iowa State University
Postdocs:	Kumar Babu Kautharapu, Iowa State University

It is recommended that the general public supplement their diet with at least 100 mg/day of omega-3 oils, such as eicosapentaenoic acid (EPA) and DHA. However, a recent article suggested that the current demand is not sustainable (Jenkins et al., 2009). Together with

concerns about possible socio-economic effects, such as the inability of local populations to continue to use fish as a food staple, and environmental contaminants in fish-derived omega-3 oils, there is a strong demand for a non-fish-derived DHA source. *M. marinus* is a promising and intriguing organism for DHA production due to its novel PKS-type enzyme. It naturally produces high amounts of DHA and would be expected to have a high tolerance for this compound. The overall goal of this project is to engineer *M. marinus* MP-1 to produce DHA at commercially viable yield and concentration. Engineering the metabolic output requires knowledge of the organism's metabolic networks and basal behavior. Phenotypic behavior and metabolic outputs can be currently measured, but the use of powerful omics tools, such as transcriptome, proteome and fluxome analysis requires the genome sequence. Therefore, the first goal of this project is to acquire a partial genome sequence by next-gen sequencing of the transcriptome in four different growth conditions.

MRI: Acquisition of a Tandem (IT-TOF) Mass Spectrometer System for Biological Research and Application

Sponsor:	National Science Foundation
Project Leader:	Ka-Yiu San, Iowa State University
Other Faculty:	George N. Bennett

The goal of this project is to establish a central mass spectrometry facility for biological research and education. To achieve this goal, a multidisciplinary research team has been assembled. The facility will provide much needed quantitative and analytical instrumentation for a number of biological projects. The team proposes to equip the facility with an ion trap time of flight (2D-nano-LC-IT-TOF) mass spectrometer.

Use of Systems Biology Approaches to Develop Advanced Biofuel-Synthesizing Cyanobacterial Strains

Sponsor:	U.S. Department of Energy
Project Leader:	Himadri Pakrashi, Washington University
Other Faculty:	Costas D. Maranas, Pennsylvania State University
Undergraduate Students:	Lou Sherman, Purdue University
Graduate Students:	Rajib Saha and Alex Verseput, Pennsylvania State University

This project investigates proteins of unknown function that regulate metabolism in plants. The work is important in that relatively little is known about the factors that influence carbon partitioning in cells, yet this understanding is crucial to achievement of high yields of desired compounds. The genes we identify in this study might function directly in yeast, or have a homolog in this organism. F urthermore, the mechanisms of metabolilc regulation may be common across multiple organisms.

Thrust 3 – Chemical Catalyst Design

A Systems Approach to Bio-Oil Stabilization

Sponsor:	U.S. Department of Energy
Project Leader:	Brent Shanks, Iowa State University
Postdocs:	Shaojun Miao and Jie Fu, Iowa State University
Other Personnel:	Yang Tang, Visiting Scholar

Due to the large number of chemical species present in biomass-derived bio-oil, a broad array of potential reactions leading to instability in bio-oil has been identified. Despite the potential for so many reactions, there is agreement that the presence of acidity, due to carboxylic acids formed during pyrolysis, and aldehydes, which are particularly reactive, in the bio-oil are key contributors to the stability issues with bio-oil. Since the acidity of the bio-oil is not only an issue for stability but also for its subsequent upgrading or use, several studies have been performed on removing acid species through their esterification with added alcohol. We are examining a different approach in which the necessary quantity of alcohol is generated by hydrogenation of the aldehydes in the bio-oil thereby obviating the need to add alcohol. In this process, aldehydes are hydrogenated to alcohols which are then esterified with the carboxylic acids. The hydrogenation/esterification coupled reaction system will be performed in a single reactor using a bifunctional hydrogenation and acid catalyst. An important advantage of this approach is that no alcohol will need to be added as it will be generated by reaction of the aldehydes. Aldehydes are more reactive than esters with respect to hydrogenation and can be achieved at a temperature of about 140°C.

Acquisition of X-ray Diffractometer for Nano-bio Materials and Earth Sciences Research

Sponsor:	National Science Foundation
Project Leader:	Abhaya Datye, University of New Mexico
Other Faculty:	Adrian Brearley, Claudia Luhrs, Darren Dunphy and Jim Connolly

We seek to acquire a powder x-ray diffractometer for advancing research and education in nano-bio materials and in earth and planetary sciences. This research grade instrument will add several new capabilities not currently available anywhere on the University of New Mexico campus. These include parallel beam focusing for thin film and small angle x-ray diffraction from mesoporous silica used for biosensors and drug delivery; monocapillary collimation optics that will enable collection of microdiffraction data from rare meteorite samples, minerals produced by microbial activity as well as ancient pottery; in-situ reaction chamber for controlled environment reaction studies of fuel cell catalysts and catalysts for conversion of biorenewables; real-time experimental studies of gas-solid reactions in geologic materials; and for study of air sensitive electrodes for lithium ion batteries. The special sample stages will allow us to perform new experiments on ferromagnetic materials of great interest for spintronics and magnetic refrigeration. The higher quality data from this instrument will allow quantitative determinations of crystal structures and phase compositions via Reitveld methods.

GOALI: Understanding Self-Assembly of Nobel Metal Alloys for Ultra Low Temperature Oxidation Catalysis

Sponsor:	National Science Foundation
Project Leader:	Abhaya Datye, University of New Mexico
Other Faculty:	B. Kiefer
Graduate Students:	Tyne Johns, University of New Mexico

The treatment of CO and hydrocarbons in the exhaust from lean burn engines is an important challenge facing the wide-spread deployment of high efficiency lean-burn automobiles. Given the increased interest in the use of bio-derived fuels, the development of low-temperature oxidation catalysts supports our nation's efforts to diversify its energy supply and reduce its dependence on foreign fossil fuels. The supplies of precious metals are limited worldwide, but there is increasing demand for clean energy. Hence, there is a need to develop more active catalysts that provide stable performance over long terms at elevated temperatures with minimal use of precious metals. In the present study, we focus on a catalyst system that preserves its composition after elevated temperature treatments, and also preserves catalytic activity over long-term operation. Understanding the principles that lead to this improved behavior could have major impact on the design of industrial catalysts which are involved at some stage in the manufacture of 90% of the products we use today.

Green Catalysis

Sponsor:	National Science Foundation
Project Leader:	L. Keith Woo, Iowa State University
Graduate Students:	B. J. Anding, Iowa State University

This project is directed towards developing a green chemistry approach to catalytic processes and sustainable, green technologies. To develop more economical and greener catalysts, environmentally friendly transition metal complexes will be explored as substitutes for the more commonly used precious and heavy metal compounds such as ruthenium and rhodium catalysts. A natural source of inspiration and insight into this issue is the world of biology. This will be used in two approaches. The first of these is simply based on the central role of iron porphyrins as the catalytic site in a variety of enzymes. This includes the cytochromes P450 and peroxidases, heme proteins involved in the catalytic transformation of a range of substrates. The wide range of heme use in nature suggested that the utility of synthetic iron porphyrin complexes in catalytic reactions is largely untapped and unappreciated.

Materials for Energy Conversion

Sponsor:	U.S. Department of Energy
Project Leader:	Plamen Atanassov, University of New Mexico
Other Faculty:	Abhaya K. Datye, University of New Mexico and Boris Kiefer
Postdocs:	Barr Halevi, University of New Mexico

The objective of this collaborative research project is to bridge bio-derived fuels with fuel cell technology as a means of electrical power generation. Biologically-derived fuels promise to be one of the most immediate implementation pathways to relieve the dependence on oil and oil imports. Fuel cells are among the core strategic technologies for energy conversion and electric power generation. There are two ways to link bio-derived fuels with fuel cell technology. One path is through development of fuel reforming and as a source of hydrogen in a way similar to the processes currently used for hydrogen production in petroleum plants. The challenges here are predominantly associated with the complex character of the bio-derived fuels, their chemical composition and the concentration of the biofuel in the feedstock. Thus, effective and selective catalyst development should be accompanied with basic research in reforming reactor engineering in order to both explore the feasibility and prepare the background for the future scale-up and scale-down efforts. Alternatively, ethanol can be used directly as a fuel in DEFC. In this case, the catalysis of ethanol oxidation presents the major challenge. Oxidative breaking of the C-C bond should be catalyzed selectively to avoid acetic acid formation, further oxidation of which presents a major obstacle. Catalytic solutions for selective oxidation steps in reforming and selective electrocatalysis of ethanol oxidation are based on the same core chemical phenomena. Therefore, we propose an integrated research program devoted to catalytic reforming and electrocatalyst development. Materials science and technology play a key role in these fuel conversion processes and lessons learned from one of them can be used as guidance for rational catalyst design in the other.

Nanostructured Catalysts for Hydrogen Generation from Renewable Feedstocks

Sponsor:	U.S. Department of Energy
Project Leader:	Abhaya K. Datye, University of New Mexico
Other Faculty:	J. Vohs, University of Pennsylvania; Y. Wang, Pacific Northwest National Labs
Undergraduate Students:	Aaron Jenkins, University of New Mexico
Graduate Students:	Jim Fitch and Eric Peterson, University of New Mexico
Postdocs:	Barr Halevi, University of New Mexico

This is a collaborative effort involving the research groups at the University of New Mexico (Datye), University of Pennsylvania (Vohs) and Pacific Northwest National Labs (Wang). The research program is directed towards the development of highly active and selective catalysts for the production of hydrogen from renewable alcohols. Our initial work has focused on the fundamental understanding of methanol conversion to hydrogen, and on associated reactions such as the water gas shift with the ultimate goal of designing advanced catalysts for converting renewable feedstocks such as bioethanol to hydrogen. The research groups have worked closely together and bring complementary and essential expertise to address DOE's grand challenge of obtaining catalytic control of molecular processes for hydrogen production from renewable sources. Our work thus far on methanol steam reforming has helped us elucidate critical factors that control the selectivity and activity of catalysts for steam reforming of methanol. While Pd by itself leads to the dehydrogenation of methanol to CO and H_2, even small amounts of Zn cause the selectivity to shift towards CO_2 and H_2 formation. We have learned that Zn is mobile and that the PdZn alloy particles form readily under reaction conditions. Zn plays many roles in this reaction system, and not all of them have been fully elucidated. First and foremost, the

presence of Zn causes a weakening of the CO bond to Pd, which helps reduce the poisoning of active sites. PdZn catalysts are consequently more active and selective to CO_2 formation. Our work also shows that the activity and selectivity are strongly dependent on the size and composition of PdZn alloy; the small amounts of CO in the product stream originate from the reverse water gas shift reaction. In summary, proper formulation of PdZn catalysts on alumina leads to active and stable catalysts that overcome many of the drawbacks of the current generation of Cu based catalysts.

National Advanced Biofuels Consortium (National Renewable Energy Lab Prime)

Sponsor:	U.S. Department of Energy
Project Leader:	Brent Shanks, Iowa State University
Graduate Students:	Yongsuck Choi, Mike Notle and Jing Zhang, Iowa State University
Postdocs:	Jie Fu, Iowa State University

This project involves developing fundamental knowledge on the pyrolysis and catalytic upgrading of biomass to intermediate chemicals that can be integrated into the existing refinery infrastructure. The upgrading portion of the work is focused on catalytic conversion of carbohydrate-derived molecules in the condensed phase.

PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials

Since this project is of particular importance to achieving the vision of the center and is integral to its strategic research and education plans, a project summary (rather than an abstract) is presented in an earlier section of Volume II.

Research Experiences for Undergraduates in Nanoscience and Microsystems

Sponsor:	National Science Foundation
Project Leader:	Abhaya K. Datye, University of New Mexico
Other Faculty:	D. Evans, University of New Mexico

The synthesis and processing of materials for the 21st century requires a new paradigm where chemistry, physics, biology, engineering and computational modeling come together. The unique aspect of our REU site program is the interdisciplinary environment, which is an essential component of materials research. We accept students majoring in materials science, ceramics, chemistry, physics and chemical, mechanical, civil and electrical engineering. They will work with faculty and research mentors in a collaborative environment that is designed to enhance interaction among the research groups. This interdisciplinary team approach reflects the environment these students will encounter as they transition to their careers in industry, academe or in national labs. The intellectual focus for the proposed REU site will come from the newly developed interdisciplinary graduate program called Nanoscience and Microsystems (NSMS). Transforming nanoscale science into systems is our unifying theme, as reflected throughout the academic and research activities, which build upon our capabilities in materials synthesis,

interrogative platforms and functional systems. The technical thrusts of the renewal REU site are: (1) Nanoscience of Biosystems, (2) Nanomaterials for Energy Conversion; and (3) Microsystems.

Responsive Catalysts

Sponsor:	U.S. Department of Energy
Project Leader:	L. Keith Woo, Andrew C. Hillier, and Robert J. Angelici, Iowa State University
Graduate Students:	Bing Hao and Nishant Sinha, University of Virginia

This Biomimetic Catalyst project focuses on the development of oligocholate foldamers that can be functionalized with various binding and catalytic groups. The Electrochemical Reduction component investigates improved methods for catalytically converting CO_2 to useful organic products. The combined effort provides unique opportunities in catalyst design that complements the electrochemical studies. Thus, biomimetic materials will also be developed to model the binding of CO_2 to metal centers and to study the details of CO_2 reduction. With specially tailored materials, it may be possible to shift the reduction potential of CO_2, when bound in a biomimetic pocket, to be sufficiently displaced from proton (or water) reduction. This would improve selectivity and the current efficiency for CO_2 reduction. In an additional approach, carbonic anhydrase mimics will be used to examine how the CO_2/HCO_3 distribution in solution may influence CO_2 reduction.

Selective Hydrogenation of Oxygenates

Sponsor:	Engineering and Physical Sciences Research Council (United Kingdom)
Project Leader:	Robbie Burch and Chris Hardacre (Queens University)
Other Faculty:	Matthew Neurock, University of Virginia
Graduate Students:	Bing Hao, University of Virginia

This work is focused on understanding the elementary steps that control the hydrogenation of saturated and unsaturated ketones and aldehydes that arise in the processing of biomass intermediates. Theory and simulation are used to understand the influence of solvent, metal, support and the molecule structure on catalytic activity and selectivity.

Selective Oxidation of Polyols

Sponsor:	National Science Foundation
Project Leader:	Abhaya K. Datye, University of New Mexico
Other Faculty:	Brent Shanks, Iowa State University; James Dumesic, University of Wisconsin; Robert Davis and Matthew Neurock, University of Virginia
Graduate Students:	David Hibbitts, University of Virginia

This work is focused on understanding the elementary steps that occur in oxidizing polyols derived from biomass to oxygenates over supported metal surface. The aim of the work is to understand the role of the metal, solution phase, promoters such as base, and molecular structure on oxidation activity and selectivity.

Structure and Function of Supported Base Catalysts

Sponsor:	U.S. Department of Energy
Project Leader:	Robert Davis, University of Virginia
Graduate Students:	Theodore Birky, Sabra Hanspal, Joseph Kozlowski, and Bhushan Zope, University of Virginia

Solid base catalysts exhibit high activities and selectivities for many kinds of reactions important for fuels and chemicals production, including transesterifications, condensations, alkylations, cyclizations, and isomerizations; however, many of these processes are carried out industrially using liquid bases as catalysts. These applications can require nearly stoichiometric amounts of the liquid base for conversion to the desired product. Replacement of liquid bases with solid base catalysts allows for easier separation from the product as well as possible regeneration and reuse. Basic solids also have the added advantages of being non-corrosive and environmentally friendly, which allows for easier disposal. The search for novel solid bases that catalyze transformations with high product selectivity, high reaction rate, and low deactivation rate is an ongoing process. Our research over the past funding cycle involved the exploration of ethanol coupling reactions on solid bases.

The Science and Engineering of Durable Ultra-Low Platinum Group Metal Catalysts

Sponsor:	Los Alamos National Labs (U.S. Department of Energy)
Project Leader:	Abhaya K. Datye, University of New Mexico
Graduate Students:	Michael Stewart, University of New Mexico
Postdocs:	Andrew De La Riva, University of New Mexico

The cost and durability of PGM cathode materials is a major barrier to the commercialization of these systems for stationary and transportation power applications. New Ultralow loading PGM cathode materials will be engineered using insights from fundamental science and tested to meet the DOE requirements for mass activity and durability. The UNM portion of this project is focused on appropriate catalyst architectures to maximize the performance of these novel catalysts. Catalyst support interactions and their effects on the durability and mass activity will be investigated.

Life Cycle Assessment

A Regional Program for Production of Multiple Agricultural Feedstocks and Processing to Biofuels and Biobased Chemicals

Sponsor:	U.S. Department of Agriculture (National Institute of Food and Agriculture - Agriculture and Food Research Initiative)
Project Leader:	Vadim Kochergin
Other Faculty:	Robert P. Anex, University of Wisconsin – Madison
Graduate Students:	Edgardo Ortiz Reyes, University of Wisconsin – Madison
Postdocs:	Rashid Rafique

This project involves a team of university and industry partners led by the LSU AgCenter, studying the production of biomass for economically viable conversion to biofuels and bioenergy using existing refinery infrastructure. Through new and existing industrial partnerships, this project will use energy cane and sweet sorghum to help reinvigorate the Louisiana sugar and chemical industries. The United States Department of Agriculture's AFRI sustainable bioenergy challenge area targets the development of regional systems for the sustainable production of bioenergy and biobased products that contribute significantly to reducing dependence on foreign oil; have net positive social, environmental, and rural economic impacts; and are compatible with existing agricultural systems. The overall project is designed to fill fundamental knowledge gaps related to sustainably growing and processing dedicated energy crops to produce drop-in biofuels and bioproducts. The science and technology being advanced will be articulated into specific biorenewable chemical pathways. The Anex research group is leading the life cycle assessment research area. It is the mission of the LCA task to evaluate the life cycle environmental impacts and measures of environmental sustainability of these biorenewable chemical and biofuel pathways. The purpose of the LCA task is to: 1) identify constraints, bottlenecks, and barriers in the pathways in order to guide and focus the research effort; 2) to provide a comparative assessment of the pathways to help determine which regional feedstock systems are most sustainable; and 3) to evaluate the environmental performance of renewable chemicals and drop-in biofuels made from dedicated energy crops grown in the south central region and processed along the specific pathways under study.

Pre-College Education

Enhancing Energy Education in Iowa

Sponsor:	Iowa Office of Energy Independence
Project Leader:	Ted Heindel, Iowa State University
Other Faculty:	Adah Leshem, Iowa State University
Graduate Students:	Jackie Baughman, Iowa State University

Overview:
Energy systems are pervasive in our society, and to address the challenges of the future, we must educate students of today in this important area. The goal of this project is to develop sustainable educational programs through Iowa State University in the area of energy and energy

systems that span K-12, undergraduate, graduate, and continuing education. These programs will be developed over the duration of this project. Upon completion, the programs will be an integral part of the ISU College of Engineering and sustainable through increased gifting (K-12), increased tuition dollars (undergraduate and graduate programs), and increased course fees (continuing education).

K-12 Outreach:

The objectives of this part of the project are to develop (i) energy demonstrations; (ii) energy education modules; (iii) curricular materials that can be used in K-12 outreach programs; and (iv) middle school teacher activities (through CBiRC) so energy education begins at the precollege level. There are many activities in which K-12 students participate. The organizers of these events are always looking for demonstrations and hands-on activities. We will develop several energy-related activities and educational packets using undergraduate students completing independent study projects or formal summer internship experiences (Program for Women in Science and Engineering, Summer Program for Enhancing Engineering Development, etc.). The activities to be developed will span all engineering disciplines and have a faculty advisor. Each proposed activity will have a small budget to be used for development costs and/or support of a student completing a summer internship experience through several of the formal programs on campus. The modules and curricular materials will also be available to teachers to be used in their classroom.

Meta!Blast: An Immersive Interactive Learning Module for Cell Biology

Sponsor:	National Institutes of Health
Project Leader:	Eve Syrkin Wurtele, Iowa State University
Other Faculty:	Adah Leshem, Iowa State University
Undergraduate Students:	Robert Goetz and Erich Langcamp, Iowa State University
Graduate Students:	P. J. Campbell, Iowa State University
Other Personnel:	William Schneller, Iowa State University

This award-winning videogame teaches about metabolic and energetic biology in the context of a cell. The project website, metablast.org, provides students and teachers with access to the game and various instructional materials. Level 1 is the basic cell and its metabolism; level 2 is the light reactions of photosynthesis. The project includes evaluation in 20 Iowa and Mississippi high schools.

Meta!Blast Awards

- 2012 - Featured at American Association for Advancement of Science Annual Meeting (http://www.aaas.org/meetings/2012/).
- 2012 - International Science and Engineering Challenge: Winner, Honorable Mention, Interactive Videogame. Science Magazine and National Science Foundation
- 2012 - International Science and Engineering Challenge Finalist, Illustration (The Cytosol). Science Magazine and National Science Foundation
- 2010 - Finalist Learning Lab (top 5% of >1,000 submissions), MacArthur Foundation
- 2010 - Second Place, Chlorofilms.

Plants in Society

Sponsor:	National Science Foundation
Project Leader:	Jonathan Wendel, Iowa State University
Other Faculty:	Adah Leshem, Iowa State University

National assessment and evaluation results suggest that K-12 science, technology, engineering and mathematics (STEM) education in our country is in crisis (National Research Council (NRC) 2005, 2009; NAEP, 2009). Despite the importance of formative education, the implementation of high quality elementary school science curricula continues to be a challenge, and elementary teachers struggle with misconceptions about what science is and how to teach it. To address this issue, the Iowa State University Plant Genomics Outreach program has created a socially relevant science workshop, *Plants in Society*, designed to provide elementary school teachers with the motivation, confidence and resources for inquiry-based curriculum and instruction development. The current study shows elementary school teachers scored significantly better on a content knowledge test covering plant and biorenewables topics after participating in the five-day workshop. A follow-up assessment revealed that, compared to other professional development experiences, teachers felt more inspired to integrate new content and pedagogical techniques into their curricula when basic principles were conveyed to them as community-centered ideas such as "going green." Additionally, ninety-four percent of the teachers reported changes in their perception of the nature of science four months after completion of the program.

University Education

A Virtual Education Center for Biorenewable Resources: Building Human Capital and Humanizing Distance Education

Sponsor:	U.S. Department of Agriculture
Project Leader:	D. Raj Raman, Iowa State University
Other Faculty:	Robert C. Brown, Thomas J. Brumm, and Jill E. Euken, Iowa State University; Sue Nokes and Czar Crofcheck, University of Kentucky; Jon Van Gerpen and Brian He, University of Idaho
Graduate Students:	Katrina Christiansen, Iowa State University
Other Personnel:	Guiping Hu, Iowa State University

Through a three-institution partnership, this project educates graduate and undergraduate students about new bio-based products and technologies. Project goals include multi-institutional development and delivery of courses; improving teaching competency of current and future faculty (doctoral candidates); and implementing, assessing, and refining a novel distance education model — the Virtual Education Center (VEC) — which allows on-site tailoring of content and mentoring, and improves the immediacy and responsiveness of the distance-ed experience. Project objectives include developing the following 3-credit courses: *Fundamentals of Biorenewable Resources*, *Biofuels*, and *Thermochemical Processing of Biomass*; developing VEC operational guidelines; and assessing the courses, VEC, and project

personnel. This project harnesses expertise across institutions to deliver coursework in areas of critical national importance. It uses existing distance education infrastructure while overcoming hurdles to distance education, such as fee collection and disbursement, variations in academic calendars and time zones, a lack of immediate feedback to students, and a lack of adjustability for local needs. The core course - *Fundamentals of Biorenewable Resources* – is part of the newly approved CBiRC Graduate Minor and is available online.

International Education

PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials

Since this project is of particular importance to achieving the vision of the center and is integral to its strategic research and education plans, a project summary (rather than an abstract) is presented in an earlier section of Volume II.

Data Management Plan

Pursuant to NSF Cooperative Agreement Financial & Administrative Terms and Conditions (FATC) Article 40, *Sharing of Findings, Data, and Other Research Products*, the NSF expects significant findings from research and education activities it supports to be promptly submitted for publication, with authorship that accurately reflects the contributions of those involved. It expects investigators to share with other researchers, at no more than incremental cost and within a reasonable time, the data, samples, physical collections and other supporting materials created or gathered in the course of the work. It also encourages awardees to share software and inventions or otherwise act to make the innovations they embody widely useful and usable.

Expected Data

The types of primary data, samples, collections, software, curriculum, and other materials that are produced in the course of the Center's activities are listed in the table at the end of this section. The plan articulates how findings, data and other research materials that have resulted fully or in part from activities supported by the NSF Engineering Directorate to CBiRC under Award No. EEC-0813570 — or by extension, through mandatory cost sharing and membership fees/revenues generated as a result of the Center's industry program — will be implemented. It outlines the rights and obligations of all parties as to their roles and responsibilities in the management and retention of said data.

Period of Data Retention

The data described herein will be managed and retained for a period of at least three years beyond the end date of the NSF Cooperative Services Agreement (currently 8/31/2013). Hence, the effective period is 9/1/2008 to 8/31/2016.

For data relevant to technology transfer, the period of retention will be 3-10 years after termination of the NSF award. Thus the period of retention would be through 8/31/2016 or 8/31/2023, depending on the technology transfer product involved.

Post-Award Monitoring

The data management plan will be based on standard university policies and practices in place at Iowa State University and/or the center's partner and collaborating institutions, and will be monitored primarily through the normal Annual and Final Report process and through evaluation of subsequent proposals.

Data Management Plan

NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)

Prepared by: Robert Anex
Thrust: Research Support – Life Cycle Assessment (LCA)
Project Title: Techno-economic Analysis of Making Hydrocarbons from Biomass-derived Sugars

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available include: Analyzed data: • Techno-economic analysis and life cycle assessment data – specifically life cycle inventories developed based on CBiRC testbeds. Metadata that define how these data are generated: • Data pedigrees that include standard quality matrices.	Dissemination approaches that will be used to make data available to others include LCI data will be uploaded to the US LCI and USDA-NAL LCA Digital Commons, where appropriate. Policies for public access and sharing include: data will be stored in the ecospold 3 data format and completed and validated datasets will be provided on request. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve publication of journal articles and reports as well as sharing of LCI data.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, include external hard disks that serve as a repository of LCI and techno-economic data sets developed by our group. We regularly back up all computers onto external hard disks and to a central computer which is used as a data "server".

Prepared by: Nancy A. Da Silva
Thrust: Research Thrust 2 – Microbial Metabolic Engineering
Project Title: T2.1B – Strain Construction and Optimization in *S. cerevisiae*
T2.2B – Strain Characterization and Optimization in *S. cerevisiae*

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: 1) DNA sequences for new GPCR variants 2) plasmids constructed and the corresponding DNA sequences 3) new yeast strains constructed 4) data obtained for experiments 5) Ph.D. dissertations, journal articles, and Powerpoint presentations	Deposited Sequence Data Sequence data for the GPCR variants will be deposited to the Genbank database and the accession number will be referenced in journal articles. Sharing of Plasmid and Yeast Strains Plasmids and other DNA fragments generated in this project will be made available free of charge to other researchers at the end of the project. A standard MTA may be required based on university policy. Technical Conferences All of this data will be presented in national meetings such as those sponsored by the American Institute of Chemical Engineers, American Chemical Society, and Engineering Conferences International (e.g., Biochemical Engineering and Metabolic Engineering Conferences). Ph.D. Dissertations and Journal Articles The results of this work will be published in appropriate scientific journals and Ph.D. dissertations.	Data Maintenance All data will be maintained in lab notebooks and stored on the lab computers with bi-weekly backup.

Prepared by: Abhaya Datye
Thrust: Research Thrust 3 – Chemical Catalyst Design
Project Title: T3.7 – Hydrothermally Stable Catalysts and Catalyst Supports

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: • TEM images, Elemental analysis, Reactivity		

Prepared by: Julie Dickerson
Thrust: Research Thrust 2 – Microbial Metabolic Engineering
Project Title: T2.5A – Bioinformatics in *E. coli*

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: • Gene expression microarray data • *E. coli* genomic Illumina sequence data • *E. coli* annotated metabolic pathway data with regulation and gene mutations Metadata that define how these data are generated: • The gene expression data follows the MIAME standards for microarray data.	Dissemination approaches that will be used to make data available to others include: • Gene expression data will be made available at public archives such as the Gene Expression Omnibus or the Short Read Archive upon publication. • Analyzed data, such as predicted gene regulators and pathway modifications are made available on public web sites upon publication acceptance. Policies for public access and sharing include making all relevant data publicly available upon publication acceptance. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve a password protected website where the data can be queried and downloaded.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, include weekly backups of all data onto long-term tape drives.

Prepared by: Peter Keeling
Program Area: Center-wide

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
Copyrightable material, including: • Reports • Books • Journal articles • Software • Databases • Sound- and video-recordings	As lead institution, ISU will be responsible for assuring that the cognizant NSF Program Officer is provided access to, either electronically or in paper form, a copy of every publication of material based on or developed under this award, clearly labeled with the award number and other appropriate identifying information, promptly after publication.	Electronic archives of data generated will be maintained using standard university policies and practices in place at Iowa State University as well as the affiliated institutions associated with CBiRC.
Technology transfer products, including: • Invention disclosures • Patent applications • Patents awarded • Licenses issued • Materials subject to Material Transfer Agreements	Any invention disclosures or patents or other technology transfer products will be formatted and managed using standard university practices in the institutional Offices of Intellectual Property.	Electronic archives of invention disclosures or patents or other technology transfer products will be maintained diligently and professionally using university and company servers and back-up hard drives.

Prepared by: Tonia McCarley (with input from Dave Beck at DBeck Creative)
Program Area: Center-wide

Expected Data	Data Formats and Dissemination	Data Storage/ Preservation of Access
Beginning this year, CBiRC will maintain non-financial data via a custom-built community web site called the Project Center. Developed in the Drupal content management system by the web development firm DBeck Creative, the Project Center allows members of CBiRC's community to access and maintain their own records of involvement in ERC work. The Project Center is password-protected, and each user must be approved by CBiRC's site administrators to gain access. Users are assigned to one or more roles which grant or restrict access to viewing and editing specific content. The important roles are as follows: • *Confirmed participant*: Basic access to the Project Center. • *PI*: Additional editorial control over certain content types; access to graduate student information. • *Leadership team*: Broader access to center-wide data and metrics. • *Content admin*: Full access to all information on the site. The Project Center tracks a broad variety of content, which has been configured to cleanly fulfill the NSF's ERC reporting requirements, while providing administrators an enhanced and detailed understanding of ERC activities. Most data are tracked longitudinally, with the aim of providing a better understanding of how the center's work has made an impact over time, and of generating reports which will help the center in its pursuit of funding beyond the ERC award. The important content types are as follows: • *Personnel profiles*: Basic information such as name, contact info, and demographics (demographics are kept private, and are for NSF reporting only). • *Personnel affiliations*: Dates and involvement levels with projects, institutions, supporting organizations, and ERC management groups. Maintained by the community, with the goal of maintaining and providing up-to-date information on each person's involvement in the center. • *Projects*: Core, associated and sponsored projects are added and maintained by the community. Individuals may specify their involvement with projects, and PIs may upload project specifics for inclusion in the annual report. • *Institutions*: Includes all academic institutions involved in the ERC, with specific information on ERC involvement level, minority programs, and demographics. Editable only by the ERC administration. • *Supporting organizations*: Includes information on industry sector, product focus, involvement level, and other metrics. Editable only by the ERC administration; many fields are hidden from regular membership. • *Thrusts, clusters and testbeds*: For tracking and aggregating ERC work by focus area. Editable only by the ERC administration. • *Publications, research presentations, outreach activities, courses, curricular products, personnel exchanges, donations,* and *tech transfers*: For tracking the various outputs and impacts of the center.	As noted, any approved member of the community may access appropriate ERC data via the Project Center, and specific users have access to more detailed reports. Data is primarily available online via web browsers, but certain reports are also downloadable in spreadsheet format. Project Center data may be downloaded by administrators pre-formatted for easy upload to the *ERCWeb* in any of the seven templated formats (thrusts and clusters, projects, personnel, organizations, institutions, project personnel, and project organizations).	The data is maintained in a secure MySQL database provided by CBiRC's web hosting service. The database is backed up to a local archive once a day, and to a second remote server once a week. Additionally, DBeck Creative creates and archives a snapshot of the entire server once a month during routine maintenance.

Prepared by: Eran Pichersky
Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering
Project Title: T1.5 – Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for the Production of Short-chain Methylketones

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: • Results of gene isolation and enzyme characterizations.	Dissemination approaches that will be used to make data available to others include scientific papers, oral and poster presentations in meetings, and submission to databases such NCBI of DNA and protein sequences. Policies for public access and sharing include those of NCBI and journals in which work is reported, as well as those of the University of Michigan. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve presentations at the regular thrust meetings, annual meetings, and through the web-based updates.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, are those of the University of Michigan.

Prepared by: D. Raj Raman
Program Area: University Education
Project Title: CBiRC REU and Graduate Minor Programs

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: • Results from surveys of student participants in REU program, and in multiple courses associated with the graduate minor. Metadata that define how these data are generated: • Policies and procedures for surveys.	Dissemination approaches that will be used to make data available to others include publication of results in posters at NSF EEC meeting, and at annual NSF site visits, and at national engineering education meetings, and in peer reviewed publications. Policies for public access and sharing include only sharing aggregate anonymous data in publications. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve anonymization of any survey data.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, include hard-drives on computers in multiple buildings on the ISU campus, specifically those of Raj Raman, Mari Kemis, and MaryAnn Moore.

Prepared by: Peter Reilly
Thrust: Research Thrust 1 – New Biocatalysts for Pathway Engineering
Project Title: T1.6 – Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems: Database Construction

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: • ThYme database of enzyme primary and tertiary structures	Dissemination approaches that will be used to make data available to others include journal articles and the publically searchable ThYme database of enzyme primary and tertiary structures. Policies for public access and sharing include journal articles and an open database. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve announcements at Center meetings, as well as articles in refereed jounals and the open database.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, include computational files, which will be stored on a dedicated server within the Iowa State University Department of Chemical and Biological Engineering.

Prepared by: Ka-Yiu San
Thrust: Research Thrust 2 – Microbial Metabolic Engineering
Project Title: T2.2A – Strain Characterization and Optimization

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: These data will consist primarily of metabolite concentrations as measured by HPLC, GC, or GC/MS, qRT-PCR enzyme activity assays. Metadata that define how these data are generated: Datasets generated from commonly accepted data acquisition software, with essential metadata presented as headers in the relevant electronic files, or included along with the indexed laboratory notebook narrative.	Dissemination approaches that will be used to make data available to others include publications and presentations. If requested, data will be made available for sharing to qualified parties by the PI, so long as such a request does not compromise intellectual property interests, interfere with publication, invade subject privacy, betray confidentiality, or precede data curation. Data that are shared will include standards and notations needed to interpret the data, following commonly accepted practices in the field. Data will be available for access and sharing as soon as is reasonably possible, normally no longer than two years after its acquisition. Policies for public access and sharing include those governed by Rice University's policies pertaining to intellectual property, record retention, and data management.. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve presentations during center related meetings and publications.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, include backing up of electronic data on hard drives in the PI's laboratory and original data notebooks will be retained in a secure location in the PI's laboratory

Prepared by: Jacqueline V. Shanks
Thrust: Research Thrust 2 – Microbial Metabolic Engineering
Project Title: T2.4A – Flux Analysis in *E. coli*
T2.4B – Flux Analysis in *S. cerevisiae*

Expected Data	Data Formats and Dissemination	Data Storage and Preservation of Access
The types of digital data to be archived and made available includes: Analyzed data: • The type of data being generated at this project is labeled metabolomic flux data. Metadata that define how these data are generated: • There are no current standards for reporting this data type. However, we give strain name, fermentation pH, volume, media, T, harvest time as a start.	Dissemination approaches that will be used to make data available to others include making results of the metabolic flux data available as spreadsheets in publication supplementary data files. Policies for public access and sharing include making data available after publications have been accepted and/or patents have been filed. Sharing and management of data with center members, institutional partners, and other major stakeholders will involve oral and poster presentations and email exchange of specific data files.	The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center's subcontractors, include Dell 650 Workstations and Dell 330 Workstations in 4122 Biorenewables Research Laboratory on the Iowa State University campus.

BRENT H. SHANKS
Steffenson Professor, Chemical & Biological Engineering Department
Iowa State University
1140L Biorenewables Research Laboratory, Ames, IA 50011
(515) 294-1985 / (515) 294-2689 (fax) / bshanks@iastate.edu

(a) Professional Preparation

Iowa State University	Chemical Engineering	B.S., 1983
California Institute of Technology	Chemical Engineering	M.S., 1985
California Institute of Technology	Chemical Engineering	Ph.D., 1988

(b) Appointments

2010 – present	Steffenson Professor, Chemical & Biological Engineering, Iowa State University
2008 – present	Director, NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2007 – 2010	Professor, Chemical & Biological Engineering, Iowa State University
1999 – 2007	Associate Professor, Chemical & Biological Engineering, Iowa State Univ.
1997 – 1999	Research Department Manager, Shell Chemical Company, Houston, TX
1988 - 1997	Research Engineer, Shell Chemical Company, Houston, TX

(c) Publications

i. Five publications most closely related to the proposed project

1. Deutsch, K.L. and Shanks, B.H., "Active Species of Copper Chromite Catalyst in C-O Hydrogenolysis of 5-Methylfurfuryl Alcohol," *J. Catal,*. **285**, 235-241 (2012).
2. Miao, S. and Shanks, B.H., "On the Mechanism of Acetic Acid Esterification over Sulfonic Acid Functionalized Mesoporous Silica," *J. Catal.*, **279**, 136-143 (2011).
3. Cinlar, B. and Shanks, B.H., "Characterization of the Acidic Sites in Organic Acid Functionalized Mesoporous Silica in the Aqueous Phase," *Appl. Catal. A: Gen.*, **396**, 76-84 (2011).
4. Hakim, S.H. and Shanks, B.H., "Synthesis and Characterization of Hierarchically Structured Aluminosilicates," *J. Mater. Chem.* **21**, 7364-7375 (2011).
5. Shanks, B.H., "Conversion of Biorenewable Feedstocks: New Challenges in Heterogeneous Catalysis," *Ind. Eng. Chem. Res.*, **49**, 10212-10217 (2010).

ii. Five other significant publications

1. Dapsens, P.Y., Hakim, S., Su, B.-L., and Shanks, B.H., "Direct Observation of Macropore Self-Formation in Hierarchically Structured Metal Oxides," *Chem. Comm.*, **46**, 8980-8982 (2010).
2. Snell, R.W., Combs, E. and Shanks, B.H., "Aldol Condensations using Bio-oil Model Compounds: The Role of Acid-Base Bi-functionality," *Catal. Today*, **53**, 1248-1253 (2010).
3. Tang, Y., Miao, S., Shanks, B.H., and Zheng, X., "Bifunctional Mesoporous Organic–Inorganic Hybrid Silica for Combined One-step Hydrogenation Esterification," *Appl. Catal A: Gen.*, **375**, 310-317 (2010).
4. Hruby, S.L. and Shanks, B.H., "Acid-Base Cooperativity in Condensation Reactions with Functionalized Mesoporous Silica Catalysts," *J. Catal.*, **263**, 181-188 (2009).
5. Hakim, S. and Shanks, B.H., "A Comparative Study of Macroporous Metal Oxides Synthesized via a Unified Approach," *Chem. Mater.* **21**, 2027-2038 (2009).

(d) Synergistic Activities

Lecturer, Energy and Materials from the Sun Summer School, Rolduc Abbey, Netherlands, June 2011
Advisory Board, Wi(PR)EM, University of Puerto Rico – Mayaguez, 2009-present
Lecturer, International Workshop on Biorenewables held in Seeon, Germany, August 2010.
Organizing Committee, NSF Workshop on Breaking the Chemical & Engineering Barriers to Lignocellulosic Biofuels, June 25-26, 2007.
Co-taught, Workshops on Biodiesel Technology, >600 national and international students and professionals, 2003-09.

(e) Collaborators & Other Affiliations

i. Collaborators & Co-Editors (past 48 months)

James Dumesic, Chemical and Biological Engineering, University of Wisconsin
Matt Neurock, Robert Davis, Chemical Engineering, University of Virginia
Abhaya Datye, Chemical Engineering, University of New Mexico
Bert Chandler, Chemistry, Trinity University
Linda Broadbelt, Chemical Engineering, Northwestern University
Robert Brown, Ted Heindel, Mechanical Engineering, ISU
George Kraus, Klaus Schmidt-Rohr, Keith Woo, Chemistry, ISU
Basil Nikolau, Biochemistry, ISU
Ka-Yiu San, Ramon Gonzalez, Chemical Engineering, Rice University
Nancy Da Silva, Chemical Engineering, U. California, Irvine
Tony Dean, Chemical Engineering, Colorado School of Mines

ii. Graduate Advisors and Postdoctoral Sponsors

James E. Bailey (deceased)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Past Students: Jason Bootsma (Ph.D. – 2006, Poet), Karl Albrecht (Ph.D. – 2008, Pacific Northwest National Laboratory), Sarah Hruby (Ph.D. – 2009, ConocoPhillips Company), Sikander Hakim (Ph.D. – 2009, University of Wisconsin), Zheng Li (Ph.D. – 2009, Center for Sustainable Environmental Technologies), Nattaporn Lohitharn (Postdoc – 2009, Logos Technologies); Shaoju Miao (Postdoc – 2010, American Scientific); Basak Cinlar (Ph.D. – 2010, DSM); Dursan Ozcan (M.S. – 2010, University of Edinburgh); Pushkaraj Patwardhan (Ph.D. – 2010, Massachusetts Institute of Technology); Pedro Ortiz-Toral (Ph.D. – 2011, Gas Technology Institute); Keenan Deutsch (Ph.D. – 2012, BASF)
Current Students: Jason Anderson, Yongsuck Choi, Michael Nolan, Michael Nolte, Ryan Snell, Tianfu Wang, Jing Zhang
Current Postdoctoral Associates: Jie Fu

BASIL J. NIKOLAU
Frances M. Craig Professor
Department of Biochemistry, Biophysics and Molecular Biology
Director, Center of Metabolic Biology; Director, W.M. Keck Metabolomics Research Laboratory;
Deputy Director, NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
Iowa State University
2210 Molecular Biology Bld, Ames, IA 50011
515-294-9423 / 515-294-0453 (fax) / dimmas@iastate.edu

(a) Professional Preparation

Massey University, New Zealand	Biochemistry/Chemistry	B.Sc., 1st Class Hon., 1977
Massey University, New Zealand	Biochemistry	Ph.D., 1982
University of California, Davis	Biochemistry	Post-doc - 1982-1983
University of Utah	Molecular Biology	Post-doc - 1983-1985

(b) Appointments

2008-present	Deputy Director, NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
2007-present	Director, Center of Metabolic Biology, ISU
2003-present	Director, W.M. Keck Metabolomics Research Laboratory, ISU
1999-2007	Director, Center for Designer Crops, ISU
1998-present	Professor, Department of Biochemistry, Biophysics and Molecular Biology, ISU
1993-1998	Associate Professor, Department of Biochemistry and Biophysics, ISU
1988-1993	Assistant Professor, Department of Biochemistry and Biophysics, ISU
1985-1988	Senior Scientist, Biotech. Department, Native Plants Inc., Salt Lake City, Utah

(c) Publications

i. Five related most closely related to the proposed project

1. Quanbeck SM, Brachova L, Campbell AA, Guan X, Perera A, He K, Rhee SY, Bais P, Dickerson JA, Dixon P, Wohlgemuth G, Fiehn O, Barkan L, Lange I, Lange BM, Lee I, Cortes D, Shuman J, Shulaev V, Huhman DV, Sumner LW, Roth MR, Welti R, Ilarslan H, Wurtele ES, **Nikolau BJ**. 2012. Metabolomics as a hypothesis-generating functional genomics tool for the annotation of *Arabidopsis thaliana* genes of "unknown function" (2012) *Frontiers in Technical Advances in Plant Science* 3:15.
2. Bais P, Moon-Quanbeck SM, **Nikolau BJ**, and Dickerson, J. A. (2012) Plantmetabolomics.org: mass spectrometry-based Arabidopsis metabolomics--database and tools update, *Nucleic Acids Research 40*, D1216-1220
3. Jin H, Song Z, **Nikolau BJ**. (2012) Reverse Genetic Characterization of two Paralogous Acetoacetyl-CoA Thiolase genes in Arabidopsis Reveals Their Importance in Plant Growth and Development, *The Plant Journal.* doi: 10.1111/j.1365-313X.2012.04942.x
4. Ding G, Che P, Ilarslan H, Wurtele, ES, and **Nikolau BJ**. (2011) Genetic dissection of methylcrotonyl CoA carboxylase indicates a complex role for mitochondrial leucine catabolism during seed development and germination, *The Plant Journal*. doi: 10.1111/j.1365-313X.2011.04893.x
5. Korte AR, Song Z, **Nikolau BJ**, and Lee YL (2011) Mass Spectrometric Imaging as a High-Spatial Resolution Tool for Functional Genomics: Tissue-Specific Gene Expression of *TT7* Inferred from Heterogeneous Distribution of Metabolites in Arabidopsis flowers, *Analytical Methods*. 4:474-481.

ii. Five other significant publications

1. Perera, MADN, Qin W, Yandeau-Nelson M, Fan L, Dixon P, **Nikolau BJ**. 2010. Biological origins of normal-chain hydrocarbons: a pathway-model based on cuticular wax analyses of maize silks. *The Plant Journal* 64: 618-632. http://www.ncbi.nlm.nih.gov/pubmed/21070415
2. Jun JH, Song Z, Liu Z, **Nikolau BJ**, Yeung ES, Lee YJ. 2010. High-spatial and high-mass resolution imaging of surface metabolites of Arabidopsis thaliana by laser desorption-ionization mass spectrometry using colloidal silver. *Analytical Chemistry* 82: 3255-3265. http://www.ncbi.nlm.nih.gov/pubmed/20235569

3. Bais P, Moon SM, He K, Leitao R, Dreher K, Walk T, Sucaet Y, Barkan L, Wohlgemuth G, Roth MR, Wurtele ES, Dixon P, Fiehn O, Lange BM, Shulaev V, Sumner LW, Welti R, **Nikolau BJ,** Rhee SY, Dickerson JA. 2010. PlantMetabolomics.org: A web portal for Plant Metabolomics Experiments. *Plant Physiology* 152: 1807-1816. http://www.ncbi.nlm.nih.gov/pubmed/20147492
4. Cha S, Song Z, **Nikolau BJ**, Yeung ES. 2009. Direct profiling and imaging of epicuticular waxes on *Arabidopsis thaliana* by laser desorption/ionization mass spectrometry using silver colloid as a matrix. *Analytical Chemistry*. 81: 2991-3000. http://www.ncbi.nlm.nih.gov/pubmed/19290666
5. Li X, Ilarslan H, Brachova L, Qian HR, Li L, Che P, Wurtele ES, **Nikolau BJ**. 2011. Reverse-genetic analysis of the two biotin-containing subunit genes of the heteromeric acetyl-coenzyme A carboxylase in Arabidopsis indicates a unidirectional functional redundancy. *Plant Physiology* 155: 293-314. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3075786/

(d) Synergistic Activities

1. Member of the scientific program organizing committee of the National Plant Lipid Cooperative (NPLC) (http://www.plantlipids.org/NPLC%202003Home.htm). Organized the 2001 and 2003 symposia, held biannually in June, at Lake Tahoe, CA.
2. Member of the International Advisory Board of the 2nd, 3rd, 4th and 5th International Congress on Plant Metabolomics, held 2003, 2004, 2006, and 2008.
3. Organizer of the 3rd International Conference on Plant Metabolomics, held in Ames, IA, June, 2004
4. Co-organizer of the 17th International Symposium on Plant Lipids, held 16-21 July 2006, at East Lansing, MI.
5. Editor of *Concepts in Plant Metabolomics* (2007). A 21-chapter book on plant metabolomics, Springer Press (ISBN-10: 1-4020-5607-9).

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Michael Beale	Beth Fatland	Jonathan Napier	Lisa Weaver
Manfred Beckmann	Ivo Feussner	John B. Ohlrogge	Ruth Welti
Raoul J. Bino	Oliver Fiehn	M.A.D.N. Perera	Randall Weselake
Edgar Cahoon	Margit Frentzen	Mike Pollard	Eve S. Wurtele
Ping Che	Jennifer A. Gray	Seung Yon Rhee	Marna D. Yandeau-Nelson
Elve Chen	Allan Green	Owen Rowland	
Julie Dickerson	Ljerka Kunst	Kazuki Saito	Hong Yao
Charles R. Dietrich	Bernd Markus Lange	Patrick S. Schnable	Edward S. Yeung
Philip Dixon	David Meinke	Vladimir Shulaev	
Peter Dörmann	Stephanie Moon	Sten Stymne	
John Draper	Rosanna Muralla	Lloyd W. Sumner	

ii. Graduate and Postdoctoral Advisors

Roger Slack, retired; Clem Hawke, deceased; Paul K. Stumpf, deceased; Daniel F. Klessig, Boyce Thompson Institute for Plant Research

iii. Thesis Advisor and Postgraduate Scholar Sponsor (past 48 months)

***PhD**--Past (15):* James Caffrey, Ping Che, Joong-Kook Choi, Tomas Diez, Geng Ding, Joel Hansen, Huanan Jin, Yuqin Jin, Linsen Liu, Ann Perera, Joel Schmidt, Jianping Song, Li Xu, Xun Wang, Tuan-Nan Wen*; Current (10):* Alexis Campbell, Daolin Cheng, Jennifer Chmielowski, Shivani Garg, Xin Guan, Fuyuan Jing, Adarsh Jose, Wenmin Qin, Lucas Showman, Bryon Upton

***MS**--Past (11):* Elve Chen, Hsiaopo Cheng, Li Wei Cui, Jennifer Gray, Terence Hung-Wa Hui, Devlina Lahiri, Ping Li, Michael Tomas McDowell, Michael Tomas McDowell, Angela L. McKean, Stephanie Moon; *Current (3):* Jason Hart, Jennifer Robinson

***Postdoctoral Fellows** --Past (8):* Laura Civardi, Beth Fatland, Vandana Mhaske, Cyril Periappuram, Cunxi Wang, Lisa M. Weaver, Lankun Wu, Wenxu Zhou*; Current (3):* Geng Ding, Huanan Jin, Zhihong Song. ***Research Associates**--Past (3):* Wei Huang, Greg Keller, Stephanie Moon Quanbeck*; Present (4):* Dr. Libuse Brachova, Dr. Marna Yandeau-Nelson, Dr. Ludmila Rizshsky, Dr. Ann Perera

25 PhD; 14 MS; 11Postdoctoral Fellows; 7 Research Associates

ROBERT P. ANEX
Professor, Biological Systems Engineering
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(a) Professional Preparation

University of California, Davis	Mechanical Engineering	B.S., 1981
University of California, Davis	Mechanical Engineering	M.S., 1983
University of California, Davis	Environmental Engineering	Ph.D., 1995

(b) Appointments

2010 – present	Professor, Biological Systems Engineering, University of Wisconsin, Madison
2009 – 2010	Professor, Agricultural & Biosystems Engineering, and Mechanical Engineering, Iowa State University
2003 – 2009	Assoc. Professor, Agricultural & Biosystems Engineering, and Mechanical Engineering, Iowa State University
2005 – 2009	Assoc. Director, Bioeconomy Institute, Iowa State University
2002 – 2003	Assoc. Professor, Aerospace & Mechanical Engineering, and Research Fellow, Institute for Science and Public Policy, University of Oklahoma
1996 – 2002	Asst. Professor, Aerospace & Mechanical Engineering, and Research Fellow, Institute for Science and Public Policy, University of Oklahoma
1989 – 1991	Senior Engineer & Section Head, Systems Control Technology, Inc., Palo Alto, CA
1983 – 1989	Research Engineer, Systems Control Technology, Inc., Palo Alto, CA

(c) Publications

i. Five publications most closely related to the proposed project

1. Kabir F. K., A. D. Patel, J. C. Serrano-Ruiz, J. A. Dumesic, R. P. Anex. 2011. Techno-economic analysis of Dimethylfuran (DMF) and Hydroxymethylfurfural (HMF) production from pure fructose in catalytic processes. *Chemical Engineering* 169(1-3): 329-38
2. Patel, A. D., J. C. Serrano-Ruiz, J. A. Dumesic, R. P. Anex. 2010. Techno-economic analysis of 5-nonanone production from levulinic acid. *Chemical Engineering Journal* 160: 311-321.
3. Anex, R. P., A. Aden, F. K. Kazi, J. Fortman, R. M. Swanson, M. M. Wright, J. A. Satrio, R. C. Brown, D. E. Daugaard, A. Platon, G. Kothandaraman, D. D. Hsu, A. Dutta. 2010. Techno-economic comparison of biomass-to-transportation fuels via pyrolysis, gasification, and biochemical pathways. *FUEL* 89 (2010): S29-S35. doi:10.1016/j.fuel.2010.07.015
4. F. K. Kazi, J. Fortman, R.P. Anex, D.D. Hsu, A. Aden, A. Dutta, G. Kothandaraman. Techno-economic comparison of process technologies for biochemical ethanol production from corn stover. *FUEL* 89 (2010): S20–S28. doi:10.1016/j.fuel.2010.01.001.
5. Anex, R.P. and A.L. Ogletree. 2006. Life Cycle Assessment of Energy-based Impacts of a Biobased Process for Producing 1,3-Propanediol. In J. Bozell and M. Patel (Eds.) *Feedstocks for the Future: Renewables for the Production of Chemicals and Materials* (pp. 222-238), London: Oxford University Press.

ii. Five other significant publications

1. Bennett, A.S. and R.P. Anex. Production, Transportation and Milling Costs of Sweet Sorghum as a Feedstock for Bioethanol Production. *Bioresource Technology*, Volume 100(4):1595-1607, February 2009.
2. Heggenstaller, A.H., R.P. Anex, M. Liebman, D.N. Sundberg, L.R. Gibson. 2008. Productivity and nutrient dynamics in bioenergy double-cropping systems. *Agronomy Journal* 100(6):1740-1748.
3. Isci, A., J. N. Himmelsbach, J. Strohl, A. L. Pometto III, D. R. Raman, R. P. Anex. Pilot scale fermentation of aqueous ammonia soaked switchgrass. *Applied Biochemistry and Biotechnology*, published online 21 August 2008.
4. Anex, R.P., L.R. Lynd, M.S. Laser, A.H. Heggenstaller, M. Liebman. 2007. Potential for enhanced nutrient cycling through the coupling of agricultural and bioenergy systems. *Crop Science Journal* 47:1327-1335.
5. Isci, A., J. N. Himmelsbach, A. L. Pometto III, D. R. Raman, R. P. Anex. 2007. Aqueous ammonia soaking of switchgrass followed by simultaneous saccharification and fermentation. *Applied Biochemistry and Biotechnology* 144(1):69-77.

(d) Synergistic Activities

Life Cycle Assessment Lead, USDA/AFRI Bioenergy CAP project, 2011-2016
Systems Analysis Lead, USDA/AFRI Corn Systems CAP project, 2011-2016.
Principal Investigator, NSF project CBET0829023, Biofuels and the Hydrologic Cycle, 2008-11.
Chair, NSF Workshop on the Land Use and Water Impacts of Biofuels, August 2009.
Associate Editor, *Intl. Journal of Life Cycle Assessment, Biotechnology for Biofuels* and *Journal of Industrial Ecology*.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Aden, A.	Natl. Renewable Energy Lab	Liebman, M.	Iowa State University
Birrel, S.	Iowa State University	Lynd, L.	Dartmouth College
Coors, J.	University of Wisconsin	Moore, K.	Iowa State University
Dumesic, J.	University of Wisconsin	Muck, R.	USDA/ARS
Hatfield, J.	USDA/NSTL	Raman, D.R.	Iowa State University
Hess, J.R.	DOE/INL	Richard, T.	University of Pennsylvania
Hinrichs, C.	University of Pennsylvania	Sheehan, J.	University of Minnesota
Hsu, D.	Natl. Renewable Engergy Lab	Shinners, K.	University of Wisconsin

ii. Graduate and Post-Doctoral Advisors

Englehardt, J. University of Miami Hubbard, M. UC-Davis Lund, J. UC-Davis

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Students

A. Isci (Ph.D. 2008, Mascoma), K. Edwards (M.S. 2008), A. Heggenstaller (Ph.D. 2009, MRI), J. Fortman (M.S. 2009), A. Patel (M.S. 2009), Y-N Wu (M.S. 2009)

Current Students

S. Khanal, Ph.D.; S. Dhungel, Ph.D., S. Gunukula, Ph.D., E. Oritz-Reyes, Ph.D., L. Gu, M.S., A. Li, M.S.

Postdoctoral Associates

Dr. F. Kabir Kazi (2008-2010), Dr. B. Gelder (2011), Dr. Rashid Rafique (current)

26 = Total number of graduate students advised and postdocs sponsored

KRISHNA S. ATHREYA
Diversity Director, CBiRC
Iowa State University
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515-294-3284 / 515-294-1269 (fax) / ksa1@iastate.edu

(a) Professional Preparation

Cotton College, Guwahati, Assam, India	Physics	B.Sc. & M. Sc., 1976
Iowa State University	Condensed Matter Physics	Ph. D. , 1986
Iowa State University	Superconductivity	1987-1989

(b) Appointments

May 2011-April 2012	Diversity Co-Director, CBiRC
March 2006-May 2010	Director, Engineering Leadership Program Adjunct Associate Professor, Materials Science and Engineering Iowa State University
Feb 2000-June 2004	Director, Women's Programs in Engineering, Cornell University
May 2001-Aug 2003	Interim director, Minority Programs in Engineering, Cornell University
March 1993-Feb 2000	Coordinator, Program for Women in Science and Engineering, Iowa State University
Aug 1987-May 1992	Temporary Assistant Professor, Department of Physics, Iowa State University
June 1988-May 1989	Post-doctoral Research Associate, Ames Laboratory, DOE, Iowa State University

(c) Publications

i. Five publications most closely related to the proposed project

1. Krishna S. Athreya, Nidhi Bhandari, Michael T. Kalkhoff, Diane T. Rover, Alexandra M. Black, Elif Eda Miskioğlu, and Steven K. Mickelson, *Work in Progress:Engineering Leadership Program: A Thematic Learning Community*, Proc. 40th ASEE/IEEE Frontiers in Education Conference, F2D-1, 2010
2. Elif Eda Miskioğlu, Krishna S. Athreya, Nidhi Bhandari, Michael T. Kalkhoff, Diane T. Rover, Alexandra M. Black, Nathan D. Meisgeier, *Engineering Leadership Program: The First Year Experience*, Proc. 40th ASEE/IEEE Frontiers in Education Conf., T4E-1, 2010
3. Michael Kalkhoff, Krishna S. Athreya, Diane Rover, Steven K Mickelson and Amy Joines, "*Highlights and Challenges of a Student Driven Co-Curricular Leadership Program*, Proc. 39th ASEE/IEEE Frontiers in Education Conf., session T4F-1, 2009
4. Krishna S. Athreya, Michael Kalkhoff, Gregory McGrath, Adam Bragg, Amy Joines, Diane T. Rover and Steven Mickelson, *Work in Progress: Engineering Leadership Program: Tracking LeadershipDevelopment of Students using Personalized Portfolios*, Proc. 38th ASEE/IEEE Frontiers in Education Conference, F3C-11, 2008.
5. Krishna S. Athreya, Diane Rover, Sarah Walter, Steven K. Mickelson, Gregory McGrath, Michael Kalkoff, Tyler Rasmussen, Gloria Starns, Rhonda Wiley-Jones, Kevin Saunders, Mack Shelley, *Work in Progress-Progression of an Engineering Leadership Program for the Future*, Proc. 37th ASEE/IEEE Frontiers in Education Conference, T2J-16, 2007.

ii. Five other significant publications

1. Ryan Legg, Mark Tekippe, Krishna S. Athreya, and Mani Mina, *Solving Multidimensional Problems Through a new Perspective: The integration of design for sustainability and engineering education,* Proc. ASEE Annual Conference, 2005
2. Yalem Teshome, Nancy Maushak and Krishna Athreya, *Attitude Toward Informal Science and Math: A Survey of boys and Girls participating in hands-on science and Math (Funtivities)*, Journal of Women and Minorities in Science and Engineering, **7,** 59 (2001).
3. Kit-Yee Daisy Fan, K. S. Athreya and R. J. Burt, *The CURIE River Basin: Introduction to Engineering in a Social Context,* Proceedings of the ASEE Annual Conference, 2001.
4. K. S. Athreya, S. C. Sanders, D. Hofreiter, and D. K. Finnemore, "Critical-Current-Free-Energy Relations in High T_c Superconductors," Phys. Rev. B **40**, 264(1990).
5. K. S. Lichtenberger, S. C. Sanders, K. S. Athreya, O. B. Hyun, and D. K. Finnemore, "Factors that Control J_c in High T_c Superconductors," Proceedings of Critical Currents in High T_c Superconductors, Karlsruhe, FRG, 1989, Cryogenics **30**, 846(1990).

(d) Synergistic Activities

President (also co-founder) *Engineers for a Sustainable World (formerly, Engineers Without Frontiers)*, www.eswusa.org, 2002-2010
Facilitator, Dialogues in Diversity course, Iowa State University, 2004-2006
Member, Action Planning Committee, Indo US Collaboration for Engineering Education (IUCEE), 2007
Special Advisor, Committee on Opportunities in Science (COOS), American Association for the Advancement of Science (AAAS), 2009-present
Board appointed member of COOS committee, AAAS, 2003-2006 and 2006-2009
Advisory Committee, *Access to Advancement: An Audio Exploration of the National Effort to Increase the Role of Women with Disabilities in Science, Technology, Engineering, and Mathematics (STEM),*WAMC Northeast Public Radio, 2008-2010

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Ms. Nell Brady	WAMC Northeast Public Radio
Mr. Glenn Busby	WAMC Northeast Public Radio
Dr. Yolanda George	American Association for the Advancement of Science
Dr. Shirley Malcolm	American Association for the Advancement of Science

ii. Graduate Advisors and Postdoctoral Sponsors (your own)

Dr. Robert N. Shelton	Executive Director, Fiesta Bowl Graduate Advisor)
Dr. Douglas Finnemore	Retired Professor Emeritus, Iowa State University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

N/A

THOMAS A. BOBIK
Associate Professor, Department of Biochemistry, Biophysics, & Molecular Biology
Iowa State University
2164 Molecular Biology Bldg., Ames, IA 50011
515-294-4165 / 515-294-0453 (fax) / bobik@iastate.edu

(a) Professional Preparation

Indiana University, Bloomington, IN	Microbiology	B.S., 1979
University of Illinois, Urbana, IL	Microbiology	M.S., 1986
University of Illinois, Urbana, IL	Microbiology	Ph.D., 1990

(b) Appointments

2009-present	Professor	Biochemistry, Biophysics and Mol. Biol. Iowa State University, Ames, IA
2004-2009	Associate Professor	Biochemistry, Biophysics and Mol. Biol. Iowa State University, Ames, IA
1995-2003	Assistant Professor	Microbiology and Cell Science University of Florida, Gainesville, FL
1990-1995	Postdoctoral Fellow	University of Utah, Salt Lake City, UT Advisor, John R. Roth
1985-1990	Graduate Student	University of Illinois, Urbana, IL Advisor, Ralph S. Wolfe

(c) Publications (47 total)

i. Five publications most closely related to the proposed project

1. Fan C, Fromm H. J., **Bobik T. A.** (2009) Kinetic and functional analysis of L-threonine kinase, the PduX enzyme of *Salmonella enterica*. J. Biol. Chem. 284, 20240-20248.
2. Beeby M., **Bobik T. A**, Yeates T. O. (2009) Exploiting genomic patterns to discover new supramolecular protein assemblies. Protein Sci. 1:69-79.
3. Fan, C. and **Bobik, T. A.** (2008) The PduX enzyme of *Salmonella* enterica is an L-threonine kinase used for coenzyme B_{12} synthesis. J. Biol Chem. 283, 11322-11329.
4. Fan, C. and **Bobik, T. A.** (2008) Functional characterization and mutational analysis of the human ATP:Cob(I)alamin adenosyltransferase. Biochemistry. 47, 2806-2813
5. Sampson, E. and **Bobik, T. A.** (2008) Bacterial microcompartments provide protection from DNA and cellular damage by reactive metabolic intermediates. J. Bacteriol. 190, 2966-2971.

ii. Five other significant publications

1. Liu, Y., Leal, N. A., Sampson, E. M., Johnson, C. L. V., Havemann, G. D. and **Bobik, T. A.** (2007) PduL is an evolutionarily distinct phosphotransacylase involved in B_{12}-dependent 1,2-propanediol degradation by *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. 185,1589-1596.
2. Leal, N. A., Olteanu, H., Banerjee, R., and **Bobik, T. A**. (2004) Human ATP:Cob(I)alamin adenosyltransferase and its interaction with methionine synthase reductase. J. Biol. Chem. 279, 47536-47542.
3. Leal, N. A., Park, S. D., Kima, P. E. and **Bobik, T. A.** (2003). Identification of the human and bovine ATP:Cob(I)alamin adenosyltransferase cDNA based on complementation of a bacterial mutant. J. Biol. Chem. 278, 9227-9234.

4. Havemann, G. D. and **T. A. Bobik**. (2003) Protein content of the polyhedral organelles involved in coenzyme B_{12}-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. 185, 5086-5095.
5. **Bobik, T. A**. and Rasche, M. E. (2001) Identification of the human methylmalonyl-CoA racemase gene based on the analysis of prokaryotic gene arrangements: implications for decoding the human genome. J. Biol. Chem. 276, 37194-37198

(d) Synergistic Activities

Dr. Bobik's specialty is bacterial genetics and physiology. He is currently conducting fundamental studies on vitamin B_{12} metabolism and bacterial microcompartments as well as applied studies on bacterial metabolic pathway engineering. The applied studies include genetic engineering of *Escherichia coli* for the production of renewable chemicals, and the construction of novel pathways for production of bioethanol and advanced biofuels.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Dr. Todd Yeates, UCLA

ii. Graduate Advisors and Postdoctoral Sponsors

Ralph S. Wolfe, Graduate advisor, University of Illinois, Urbana-Champaign
John R. Roth, Post-doctoral advisor, University of California, Davis

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 5 years)

Zhu, Huilin, PhD student, 2012	WuXi AppTec (pharmaceuticals)
Gogerty, David, PhD student, 2012	ISU
Cheng, Shouqiang, PHD, 2011	Northwestern University, Chicago, IL
Fan, Chenguang, PhD, 2010	ISU
Liu, Yiu, MS student, 2009	UC Berkeley
Agarkar, Netra, MS student, 2008	NA
Sinha, Sharmistha, Post-doctoral, current	ISU
Fan, Chenguang, Post-doctoral, current	ISU
Gogerty, David, Post-doctoral, current	ISU
Cheng, Shouqiang, Post-doctoral, 2012	Northwestern University, Chicago, IL
Hennen-Bierwagon, Tracie, Post-doctoral, 2009	ISU

11 = Total number of graduate students advised and postdocs sponsored last 5 years.

NANCY A. DA SILVA
Professor, Department of Chemical Engineering and Materials Science
University of California, Irvine
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(a) Professional Preparation

University of Massachusetts	Chemical Engineering	B.S., 1982
California Institute of Technology	Chemical Engineering	M.S., 1985
California Institute of Technology	Chemical Engineering	Ph.D., 1988

(b) Appointments

2004 – present	Professor, Biomedical Engineering (joint appointment), UC Irvine
2000 – present	Professor, Chemical and Biochemical Engineering, UC Irvine
1994 – 2000	Assoc. Professor, Chemical and Biochemical Engineering, UC Irvine
1988 – 1994	Asst. Professor, Chemical and Biochemical Engineering, UC Irvine

(c) Publications

i. Five publications most closely related to the proposed project.

1. S. Srikrishnan, A. Randall, P. Baldi, N.A. Da Silva*. 2012. Rationally selected single-site mutants of the *Thermoascus aurantiacus* endoglucanase increase hydrolytic activity on cellulosic substrates. *Biotechnology and Bioengineering*. DOI: 10.1002/bit.24414 (in press).
2. N.A. Da Silva*, S. Srikrishnan. 2012 (in press). Minireview: Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*. *FEMS Yeast Research*. **12**, 197-214.
3. F. Fang, K. Salmon, M.W.Y. Shen, K.A. Aeling, E. Ito, B. Irwin, U. Tran, G.W. Hatfield, N.A. Da Silva*, S. Sandmeyer*. 2010. A vector set for systematic metabolic engineering in *Saccharomyces cerevisiae*. *Yeast*, **28**, 123-136.
4. S.M. Ma, J.W.-H. Li, J.W. Choi, H. Zhou, K.K.M. Lee, V.A. Moorthie, X. Xie, J.T. Kealey, N.A. Da Silva, J.C. Vederas*, Y. Tang*. 2009. Complete reconstitution of a highly reducing iterative polyketide synthase. *Science*, **326**, 589-592.
5. K.M. Lee, N.A. Da Silva*, J.T. Kealey*. 2009. Determination of the extent of phosphopantetheinylation of polyketide synthases expressed in *Escherichia coli* and *Saccharomyces cerevisiae*. *Analytical Biochemistry*, **394**, 75-80.

ii. Five other significant publications

1. W. Lee and N.A. Da Silva*. 2006. Application of sequential integration for metabolic engineering of 1,2-propanediol production in yeast. *Metabolic Engineering*, **8**, 58-65
2. S.C. Mutka, S.M. Bondi, J.R. Carney, N.A. Da Silva, and J.T. Kealey*. 2006. Metabolic pathway engineering for complex polyketide biosynthesis in *Saccharomyces cerevisiae*. *FEMS Yeast Research*, **6**, 40-47.
3. S.W.P. Chan, S.-P. Hung, S.K. Raman, G.W. Hatfield, R.H. Lathrop, N.A. Da Silva*, S.-W. Wang*. 2010. Recombinant human collagen and biomimetic variants using a de novo gene optimized for modular assembly. *Biomacromolecules*. **11**, 1460-1469.
4. D. Shah, M.W.Y. Shen, W. Chen, N.A. Da Silva*. 2010. Enhanced arsenic accumulation in *Saccharomyces cerevisiae* overexpressing transporters Fps1p or Hxt7p. *Journal of Biotechnology*. **150**, 101-107.

5. D.C. Pecota, V. Rajgarhia, and N.A. Da Silva*. 2007. Sequential gene integration for the metabolic engineering of *Kluyveromyces marxianus*. *J. Biotechnol.*, **127**, 408-416.

(d) Synergistic Activities

UCI ADVANCE Program (Sponsored by the NSF ADVANCE Program): Service as Equity Advisor for The Henry Samueli School of Engineering working with the Dean, Department Chairs, Search Committees on the recruitment, retention, and advancement of women faculty. Responsibilities include the development of Assistant Professor mentoring programs, and organizing panels and meetings for women students.
Service on editorial boards for *Journal of Biotechnology* and *Applied Biochemistry and Biotechnology*.
BioEMB: Bioengineering Educational Materials Bank, Member of Advisory Board.
Headed curriculum development and new major initiatives for the department. Took the lead in outlining and implementing curriculum changes for the undergraduate Chemical Engineering major. Led ABET accreditation activities.

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

Pierre Baldi, Professor, Arlo Randall, Ph.D.; University of California, Irvine
Rachel Chen, Professor; Georgia Tech
Wilfred Chen, Professor; University of California, Riverside
Lucília Domingues, Professor; Universidade do Minho, Portugal
G. Wesley Hatfield, Professor; University of California, Irvine
James E. Kealy, Ph.D., Sarah Mutka, Ph.D., formerly at Kosan Biosciences
Richard H. Lathrop, Professor; University of California, Irvine
Kirsty Salmon, Ph.D.; Verdezyne
Suzanne Sandmeyer, Professor; University of California, Irvine
Yi Tang, Professor; University of California, Los Angeles
Sheryl Tsai, Professor; University of California, Irvine
John C. Vederas, Professor; University of Alberta, Canada
Szu-Wen Wang, Assistant Professor; University of California, Irvine

ii. Graduate Advisor

James E. Bailey (deceased)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Current Postgraduate-Scholar:
Abeer Jabalah

Past Students:
Manely Kouhssari (M.S. – 2008), Dhawal Shah (Ph.D. – 2007), Ka Kit Michael Lee (Ph.D. – 2006)

Current Students:
Sam Wei Polly Chan, Jin Wook Choi, Christopher Leber, Michael W.Y. Shen, Sneha Srikrishnan, Ruben Fernandez Moya, Richard Que

30 = Total Number of Graduate Students Advised and Postdoctoral Associates Sponsored

ABHAYA K. DATYE
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(a) Professional Preparation

Indian Institute of Technology	Chemical Engineering	B.S., 1975
University of Cincinnati	Chemical Engineering	M.S., 1980
University of Michigan	Chemical Engineering	Ph.D., 1984

(b) Appointments

2010 – present	Regents Professor
2008 – present	Distinguished Professor
2007 – present	Director of the Nanoscience & Microsystems graduate program, UNM
1994 – present	Director, Center for Micro-engineered Materials (CMEM)
2002 – 2007	Associate Chair, Department of Chemical and Nuclear Engineering
2004 – 2007	Site Director, NSF/IUCRC Ceramic and Composite Materials Center
1999 – 2004	Executive Director, NSF/IUCRC Ceramic and Composite Materials Cntr.
1994 – 1999	Director NSF/IUCRC Center for Microengineered Materials
1984 – present	Chemical & Nuclear Engineering, University of New Mexico
1976 – 1978	Hindustan Organic Chemicals, Rasayani, India, Scientific Officer
1975 – 1976	Hindustan Lever Ltd., Research Center, Bombay, India, Research Asst.

(c) Publications

i. Five publications most closely related to the proposed project

1. Q. Xu, K.C. Kharas, B.J. Croley, and A.K. Datye, *The Sintering of Supported Pd Automotive Catalysts.* Chemcatchem, 2011. **3**(6): p. 1004-1014.
2. Y. Tang, S. Miao, H.N. Pham, A. Datye, X. Zheng, and B.H. Shanks, *Enhancement of Pt catalytic activity in the hydrogenation of aldehydes.* Applied Catalysis A-General, 2011. **406**(1-2): p. 81-88.
3. G.K. Smith, S. Lin, W.Z. Lai, A. Datye, D.Q. Xie, and H. Guo, *Initial steps in methanol steam reforming on PdZn and ZnO surfaces: Density functional theory studies.* Surface Science, 2011. **605**(7-8): p. 750-759.
4. H.N. Pham, Y.J. Pagan-Torres, J.C. Serrano-Ruiz, D. Wang, J.A. Dumesic, and A.K. Datye, *Improved hydrothermal stability of niobia-supported Pd catalysts.* Applied Catalysis A-General, 2011. **397**(1-2): p. 153-162.
5. E.J. Peterson, B. Halevi, B. Kiefer, M.N. Spilde, A.K. Datye, J. Peterson, L. Daemen, A. Llobet, and H. Nakotte, *Aerosol synthesis and Rietveld analysis of tetragonal ($\square_1$) PdZn.* Journal of Alloys and Comp., 2011. **509**(5): p. 1463-1470.

ii. Five other significant publications

1. Y.J. Pagan-Torres, J.M.R. Gallo, D. Wang, H.N. Pham, J.A. Libera, C.L. Marshall, J.W. Elam, A.K. Datye, and J.A. Dumesic, *Synthesis of Highly Ordered Hydrothermally Stable Mesoporous Niobia Catalysts by Atomic Layer Deposition.* ACS Catalysis, 2011. **1**(10): p. 1234-1245.
2. S.E. Davis, L.R. Houk, E.C. Tamargo, A.K. Datye, and R.J. Davis, *Oxidation of 5-hydroxymethylfurfural over supported Pt, Pd and Au catalysts.* Catalysis Today, 2011. **160**(1): p. 55-60.

3. M. Chia, Y.J. Pagan-Torres, D. Hibbitts, Q. Tan, H.N. Pham, A.K. Datye, M. Neurock, R.J. Davis, and J.A. Dumesic, *Selective Hydrogenolysis of Polyols and Cyclic Ethers over Bifunctional Surface Sites on Rhodium-Rhenium Catalysts.* Journal Of The American Chemical Society, 2011. **133**(32): p. 12675-12689.
4. S.R. Challa, A.T. Delariva, T.W. Hansen, S. Helveg, J. Sehested, P.L. Hansen, F. Garzon, and A.K. Datye, *Relating Rates of Catalyst Sintering to the Disappearance of Individual Nanoparticles during Ostwald Ripening.* Journal Of The American Chemical Society, 2011. **133**(51): p. 20672-20675.
5. P.D. Burton, T.J. Boyle, and A.K. Datye, *Facile, surfactant-free synthesis of Pd nanoparticles for heterogeneous catalysts.* Journal of Catalysis, 2011. **280**(2): p. 145-149.

(d) Synergistic Activities

As director of a NSF/Research Experiences for Undergraduates Site Program, I have organized a summer program (since 1995) for students from other universities to spend 10 weeks on campus working with researchers at our center. During the summers of 1999- 2001, we also brought 3 high school teachers each year into our summer program via the RET (Research Experiences for Teachers) program funded by NSF. As the site director for the NSF/EPSCOR program in Nanoscience at UNM, I have helped organize an outreach program that involves workshops aimed at high school teachers. We secured funding from a foundation to provide kits that teachers can take back to their classes. We have developed a new interdisciplinary curriculum in Nanoscience and Microsystems, as part of the NSF/IGERT program.

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)
Larry Allard, Jeff Brinker, Neil Coville, Bob Davis, Jim Dumesic, Paul Hansen, Stig Helveg, Charles Kappenstein, Karl C. C. Kharas, Jeff Miller, Matt Neurock, Robert Schløgl, Brent Shanks, John Vohs, Yong Wang

ii. Graduate Advisor
Robert Lemlich – University of Cincinnati, Johannes Schwank – University of Michigan

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)
Students Graduated (27 Ph. D, 34 M. S) Recent graduates:
Patrick Burton, PhD 2011, Adam Tsosie, MS 2011, Elena Berliba Vera, MS 2011, Noel Dawson, MS 2011

Present Research group: Eric Petersen, Angelica Sanchez, Jonathan Paiz, Tyne Johns, Jim Fitch, Michael Stewart *undergrad students* Lisa Lowery, Valerie Ashbacher, Amanda Anderson, Aaron Jenkins, Johnny Nogales Current Postdoctoral Associates: Barr Halevi, Hien Pham, Andrew DeLaRiva, Haifeng Xiong

ROBERT J. DAVIS
Earnest Jackson Oglesby Professor, Department of Chemical Engineering
University of Virginia
102 Engineers' Way, Charlottesville, VA 22904-4741
(434) 924-6284 / (434) 982-2658 (fax) / rjd4f@virginia.edu

(a) Professional Preparation

Virginia Tech	Chemical Engineering	B.S., 1985
Stanford University	Chemical Engineering	M.S., 1987
Stanford University	Chemical Engineering	Ph.D., 1989
University of Namur, Belgium	Chemistry	Postdoc, 1989-1990

(b) Appointments

2009 – Present	Earnest Jackson Oglesby Professor, Chem. Engr., Univ. of Virginia
2002 – 2011	Professor and Chair, Chemical Engineering, University of Virginia
1996-2002	Associate Professor, Chemical Engineering, University of Virginia
1990-1996	Assistant Professor, Chemical Engineering, University of Virginia

(c) Publications

i. Five publications most closely related to the proposed project

1. S.E. Davis, B.N. Zope and R.J. Davis "On the mechanism of selective oxidation of 5-hydroxymethylfurfural to 2,5-furandicarboxylic acid over supported Pt and Au catalysts," *Green Chem.* **14** (2012) 143-147.
2. S.E. Davis, L.R. Houk, E.C. Tamargo, A.K. Datye, and R.J. Davis, "Oxidation of 5-Hydroxymethylfurfural over Supported Pt, Pd and Au Catalysts," *Catalysis Today* **160** (2011) 55-60.
3. M. Chia, Y.J. Pagán-Torres, D. Hibbitts, Q. Tan, H.N. Pham, A.K. Datye, M. Neurock, R.J. Davis, and J.A. Dumesic, "Selective hydrogenolysis of polyols and cyclic ethers over bifunctional surface sites on rhodium-rhenium catalysts," *J. Am. Chem. Soc.* **133** (2011) 12675-12689.
4. B.N. Zope and R.J. Davis, "Inhibition of Gold and Platinum Catalysts by Reactive Intermediates Produced in the Selective Oxidation of Alcohols in Liquid Water," *Green Chem.* **13** (2011) 3484-3491.
5. B.N. Zope, D.D. Hibbitts, M. Neurock and R.J. Davis, "Reactivity of the Gold-Water Interface during Selective Oxidation Catalysis" *Science* **330** (2010) 74-78.

ii. Five other significant publications

1. O.M. Daniel, A. DeLaRiva, E.L. Kunkes, A.K. Datye, J.A. Dumesic and R.J. Davis, "X-ray Absorption Spectroscopy of Bimetallic PtRe Catalysts for Hydrogenolysis of Glycerol to Propanediols" *ChemCatChem* **2** (2010) 1107-1114.
2. B.N. Zope and R.J. Davis, "Influence of Reactor Configuration on the Selective Oxidation of Glycerol over Au/TiO_2" *Topics in Catalysis*, **52** (2009) 269-277.
3. E.P. Maris and R.J. Davis, "Hydrogenolysis of Glycerol over Carbon-supported Ru and Pt Catalysts," *J. Catal.* **249** (2007) 328-337.
4. W.C. Ketchie, Y.-L. Fang, M.S. Wong, M. Murayama, and R.J. Davis, "Influence of Gold Particle Size on the Aqueous-Phase Oxidation of Carbon Monoxide and Glycerol," *J. Catal.* **250** (2007) 94-101.
5. W.C. Ketchie, M. Murayama, and R.J. Davis, "Selective Oxidation of Glycerol over Carbon-supported AuPd Catalysts," *J. Catal.* **250** (2007) 264-273.

(d) Synergistic Activities

Chair of Gordon Conference on Catalysis, 2006.
Chair of Catalysis Programming of the AIChE, 2002-03 and Director of Catalysis and Reaction Engineering Division of AICHE, 2006-2008.
Chair of US Government panel conducting an international assessment of "Catalysis by Nanostructured Materials" 2007-2009.
Director-at-Large of the North American Catalysis Society, 2009-Present
Editorial Boards: Applied Catalysis A: General, 1998-2003; Applied Catalysis B: Environmental, 2004-2011; Journal of Molecular Catalysis A: Chemical, 2007-Present; ChemCatChem Heterogeneous, Homogeneous and Biocatalysis, 2009-Present; Journal of Catalysis, 2009-Present; ACS Catalysis, 2011-Present

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Pradeep Agrawal	Georgia Tech
Abhaya Datye	University of New Mexico
James Dumesic	University of Wisconsin
Chris Jones	Georgia Tech
M. Douglas Levan	Vanderbilt University
P.J. Ludovice	Georgia Tech
C.D. Sherrill	Georgia Tech
David Sholl	Georgia Tech
Marcus Weck	New York University
Michael Wong	Rice University
Daniela Ferrari	Dow Chemical Company
David Barton	Dow Chemical Company

ii. Graduate and Postdoctoral Advisors

Michel Boudart	Stanford University
Eric Derouane	University of Namur

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Bhushan Zope (Ph.D. – 2011, Applied Materials), Makarand Gogate (Postdoc – 2010, unknown), Yuanzhou Xi (Ph.D. – 2010, Postdoc – 2010, Cummins), Surbhi Jain (Ph.D. – 2008, ExxonMobil), William Ketchie (Ph.D. – 2007, UOP), Erin Maris (Ph.D. – 2007, Chevron)

31 = Total number of graduate students advised and postdocs sponsored.

JULIE A. DICKERSON
Associate Professor, Electrical and Computer Engineering Department
Iowa State University
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(515) 294-7705/(515) 294-8432 (fax)/julied@iastate.edu

(a) Professional Preparation

University of California, San Diego	Electrical Engineering	B.S., 1983
University of Southern California	Electrical Engineering	Ph.D., 1993

(b) Appointments

2011– present	Program Officer, National Science Foundation, BIO Directorate, Divition of Biological Infrastructure
2002 –present	Associate Professor, Electrical & Computer Engineering, Iowa State Univ.
1995 – 2002	Assistant Professor, Electrical & Computer Engineering, Iowa State Univ.
1994	Research Associate, Electrical Engineering, University of Southern California
1991 – 1993	Research Assistant, Electrical Engineering, University of Southern California
1988 – 1991	Senior Staff Engineer, Martin Marietta Space Systems
1983 – 1990	Member of the Technical Staff, Hughes Aircraft Corporation

(c) Publications

i. Five publications most closely related to the proposed project

1. Van Hemert, J.L., J.A. Dickerson, "Discriminating response groups in metabolic and regulatory pathway networks", Bioinformatics, Accepted December 2011, to appear.
2. Fu, Y., L.R. Jarboe, J.A. Dickerson, "Reconstructing genome-wide regulatory network of *E. coli* using transcriptome data and predicted transcription factor activities," *BMC Bioinformatics,* **12**:233, 2011, doi:10.1186/1471-2105-12-233.
3. P. Bais, S.M. Moon-Quanbeck, B.J. Nikolau, J.A. Dickerson, "Plantmetabolomics.org: mass spectrometry-based Arabidopsis metabolomics," *Nucleic Acids Research,* 2012 Database Issue, Early Access November 11, 2011, doi: 10.1093/nar/gkr1006.
4. P Bais, SM. Moon, K He, R Leitao, K Dreher, T Walk, Y Sucaet, L Barkan, G Wohlgemuth, MR. Roth, ES Wurtele, P Dixon, O Fiehn, B.M Lange, V Shulaev, LW. Sumner, R Welti, BJ. Nikolau, S Rhee, and JA. Dickerson, "PlantMetabolomics.org: A Web Portal for Plant Metabolomics Experiments," *Plant Physiology*, pp. 109, **DOI:10.1104/pp.109.151027,** Feb. 10, 2010.
5. Grimplet, J., Cramer, G.R., Dickerson, J.A., Mathiason, K., Van Hemert, J. and Fennell, A.Y. (2009) VitisNet: "Omics" Integration through Grapevine Molecular Networks. *PLoS One*, **4**, e8365.

ii. Five other significant publications

1. Mao, L., Van Hemert*, J., Dash, S. and Dickerson, J. (2009) Arabidopsis gene co-expression network and its functional modules. *BMC Bioinformatics*, **10**, 346.
2. Fiehn O, Sumner LW, Rhee SY, Ward J**,** Dickerson J**,** Lange BM, Lane G, Roessner U, Last R, Nikolau B (2007) Minimum reporting standards for plant biology context information in metabolomics studies. Metabolomics **3**: 195-201.
3. S.Y. Rhee, J.A. Dickerson, D. Xu, "Bioinformatics and Its Applications in Plant Biology," Annual Review of Plant Biology, **57**, 335-359, 2006.

4. Pan Du*, Jian Gong*, Eve Syrkin Wurtele, and J.A. Dickerson**,** "Modeling Gene Expression Networks using Fuzzy Logic," Special issue of *IEEE Transactions on Systems, Man and Cybernetics, Part B,* **35**(6):1351-1359, 2005.
5. Yuting Yang*, Levent Engin, Eve Syrkin Wurtele, Carolina Cruz-Neira, J.A. Dickerson, "Integration of metabolic networks and gene expression in virtual reality," *Bioinformatics*, **21**: 3645-3650.

(d) Synergistic Activities

Interdisciplinary Training in Bioinformatics: Mentored twenty IGERT fellows in lab rotations for computational biology, seven students were female; eight were domestic students. Mentored high school interns in computational biology, both students were female and underrepresented minorities.

Curriculum Development: Developed a new course on Systems Biology for the graduate program in Bioinformatics. Developed new sophomore-level course in signals and systems with labs featuring problems in computational biology.

Mentoring of underrepresented undergraduate students: Mentored two female, minority students (Alicia Guidry and Machele Lugo) Bioinformatics and Computational Biology NSF/NIH Summer Institute, 2005. Ms. Guidry is a MS student in CS at Texas A&M. Rien Beall, minority student for a project on graph-based clustering 2006. Mr. Beall is now a MS student at ISU.

Biology Education, grades 6-12: Developing the Meta!Blast video game for teaching plant cell biology and metabolism to middle and high school students. Meta!Blast features accurate models of cellular organelles and proteins from recent imaging studies and the protein databank (PDB).

Organization of Workshops or Special Courses (last 4 years): NIH/NSF Bioinformatics Summer Institute (2006-Present); 18th Annual GFST Symposium: Systems Biology: Integrative, Comparative, and Multi-Scale Modeling (2009).

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

D. Berleant,	Univ. Arkansas	D Reiners	Univ. of Louisiana
G. Cramer	Univ. Nevada Reno	SY Rhee	The Carnegie Institute
C. Cruz-Neira	Univ. of Louisiana	K.Y. San	Rice
O. Feihn	UC Davis	V. Shulaev	Virginia Tech
A. Fennell	South Dakota State Univ.	L Sumner	Noble Institute
R. Gonzalez	Rice	D. Xu	Univ. of Missouri-Columbia
M. Lange	University of Washington	R. Welti	Kansas State Univ.
D. Marshall	Scottish Crop Res. Institute		

ii. Graduate and Postdoctoral Advisors

Bart Kosko, Univ. of Southern California, Petros Ioaunnou, Univ. of Southern California

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Past Students

Sudhansu Dash (Iowa State Univ.), Pan Du (Northwestern Univ.), Joset Etzel (Univ. Medical Center Groningen, Netherlands, Caltech), Shubha Kher (Arkansas State Univ.), Linyong Mao (Iowa State Univ.), Yuting Yang (Intel), Ming Jia (Google), Preeti Bais (Stemina), Wengang Zhou and John Van Hemert (Pioneer)

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University of Wisconsin
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(a) Professional Preparation

University of Wisconsin	Chemical Engineering	B.S., 1971
Stanford University	Chemical Engineering	M.S., 1972
Stanford University	Chemical Engineering	Ph.D., 1974

(b) Appointments

1/96 - present	Steenbock Professor, Chemical Engineering Department, University of Wisconsin (UW) – Madison
1/98 - 7/00	Chair, Chemical Engineering Department, UW – Madison
1/89 - 1996	Milton and Maude Shoemaker Professor, UW – Madison
1/93 - 7/95	Chair, Chemical Engineering Department, UW – Madison
9/92 -12/92	Acting Chair, Chemical Engineering Department, UW – Madison
6/89 - 9/92	Associate Chair, Chemical Engineering Department, UW – Madison
6/82 - 12/88	Professor, Chemical Engineering, UW – Madison
6/79 - 6/82	Associate Professor, Chemical Engineering, UW – Madison
1/76 - 6/79	Assistant Professor, Chemical Engineering, UW – Madison

(c) Publications (~370 total)

i. Five publications most closely related to the proposed project

1. Bifunctional Solid Catalysts for the Selective Conversion of Fructose to 5-Hydroxymethylfurfural, *Topics in Catalysis* **53**, 1185 (2010), A. J. Crisci, M. H. Tucker, S. L. Scott, and J. A. Dumesic.
2. Production of 2,5-dimethylfuran from biomass-derived carbohydrates for liquid transportation fuels, *Nature* **447**, 982 (2007), Y. Román-Leshkov, C. J. Barrett, Z. Y. Liu, and J. A. Dumesic.
3. Catalytic Conversion of Biomass to Biofuels, *Green Chemistry* **12**, 1493 (2010), Jesse Q. Bond, David Martin Alonzo, and J. A. Dumesic.
4. Selective hydrogenolysis of polyols and cyclic ethers over bifunctional surface sites on rhodium-rhenium catalysts, *Journal of the American Chemical Society* **133**, 12675 (2011), Mei Chia, Yomaira J. Pagán-Torres, David Hibbitts, Qiaohua Tan, Hien N. Pham, Abhaya K. Datye, Matthew Neurock, Robert J. Davis, and J. A. Dumesic.
5. Integrated catalytic system to convert γ-valerolactone to liquid alkenes for transportation fuels, *Science* **327**, 1110 (2010), J. Q. Bond, D. Martin-Alonso, R. M. West, D. Wang and J. A. Dumesic.

ii. Five other significant publications

1. Inter-conversion between γ-valerolactone and pentenoic acid combined with decarboxylation to form butene over silica/alumina, *Journal of Catalysis* **281**, 290 (2011), Jesse Q. Bond, Dong Wang, David Martin Alonso, and J. A. Dumesic.

2. Production of Biofuels from Cellulose and Corn Stover Using Alkylphenol Solvents, *Chemistry and Sustainability* **4**, 1078 (2011), David Martin Alonso, Stephanie G. Wettstein, Jesse Q. Bond, Thatcher W. Root and James A. Dumesic.
3. Production of liquid hydrocarbon fuels by catalytic conversion of biomass-derived levulinic acid, *Green Chemistry* **7**, 1755 (2011), Drew J. Braden, Carlos A. Henao, Jacob Heltzel, Christos C. Maravelias and James A. Dumesic.
4. Liquid-phase catalytic transfer hydrogenation and cyclization of levulinic acid and its esters to γ-valerolactone over metal oxide catalysts, *Chemical Communications* 47(44), 12233 (2011), Mei Chia and James A. Dumesic.
5. Synthesis of highly ordered hydrothermally stable mesoporous niobia catalysts by atomic layer deposition, *ACS Catalysis* **1**, 1234 (2011), Yomaira J. Pagán-Torres, Jean Marcel R. Gallo, Dong Wang, Hien N. Pham, Joseph A. Libera, Christopher L. Marshall, Jeffrey W. Elam, Abhaya K. Datye, and James A. Dumesic.

(d) Synergistic Activities

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

ii. Graduate Advisors and Postdoctoral Sponsors

Michael Boudart, Standford University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)*

Yomaira Pagan	September 2011	Dow Chemical
Mark Tucker	June 2011	BP
Jesse Bond*	July 2011	Syracuse University
Drew Braden	August 2010	BP
Juan Carlos Serrano-Ruiz*	October 2009	University of Córdoba
Ed Kunkes	August 2009	Fritz Haber Institute
Ryan West	June 2009	Proctor and Gamble
Chris Barrett	August 2008	General Foods
Yuriy Roman-Leshkov	August 2008	MIT
Dante Simonetti	July 2008	UOP

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Departments of Chemical & Biomolecular Engineering and Bioengineering
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(a) Professional Preparation

Central University of Las Villas, Cuba	Chemical Engineering	B.Sc., 1993
Catholic University of Valparaiso, Chile	Biochemical Engineering	M.Sc., 1999
University of Chile, Chile	Chemical Engineering	Ph.D., 2001
University of Florida	Microbiology &Cell Science	PostDoc, 2001-2002

(b) Appointments

2011–Present	Associate Professor, Dept. Chemical & Biomolecular Eng., Dept. Bioengineering, Rice University, Houston, Texas.
2005–2011	William W. Akers Assistant Professor, Dept. Chemical & Biomolecular Eng., Dept. Bioengineering, Rice University, Houston, Texas.
2002–2005	Assistant Professor, Depts. of Chemical & Biological Eng. and Food Science & Human Nutrition, Iowa State University, Ames, Iowa.
2001–2002	Postdoctoral Associate, Dept. Microbiology & Cell Science, Univ. of Florida, Gainesville, Florida.
1994–1995	Process Engineer , Marcelo Salado Sugar Mill (Formerly, Reforma Sugar Mill), MINAZ (Cuba's Sugar Ministry), Caibarien, Cuba.
1993–1996	Research Associate and Lecturer, Center for Processes Analysis, Dept. Chemical Eng., Central University of Las Villas, Santa Clara, Cuba.

(c) Publications

i. Five publications most closely related to the proposed project

1. Cintolesi, A., Clomburg, J.M., Rigou, V., Zygourakis, K., and Gonzalez, R. (2012). Quantitative analysis of the fermentative metabolism of glycerol in *Escherichia coli*. *Biotechnol. Bioeng. 109 (1): 187-198*.
2. Posada, J. A., Cardona, C. A., Gonzalez, R. (2012). Analysis of the production process of optically pure D-lactic acid from raw glycerol using engineered *Escherichia coli* strains. *Appl. Bioch. Biotechnol. 166 (3): 680-699*.
3. Dellomonaco, C., Clomburg, J.M., Miller, E.N., and Gonzalez, R. (2011). Engineered reversal of the β-oxidation cycle for the synthesis of fuels and chemicals. *Nature 476, 355-359*.
4. Clomburg, J., and Gonzalez, R. (2011). Metabolic engineering of Escherichia coli for the production of 1,2-propanediol from glycerol. *Biotechnol. Bioeng. 108 (4): 867-879*.
5. Zhu, H., Gonzalez, R., and Bobik, T.A. (2011). Co-production of acetaldehyde and hydrogen from glucose fermentation by *Escherichia coli*. *Appl. Environ. Microbiol. 77 (18): 6441-6450*.

ii. Five other significant publications

1. Berrios, J., Altamirano, C., Osses, N., and Gonzalez, R. (2011). Continuous CHO cell cultures with improved recombinant protein productivity by using mannose as carbon source: metabolic analysis and scale-up simulation. *Chem. Eng. Sci. 66 (11): 2431-2439*.
2. Choudhary, M. K., Moon, J. Y., Gonzalez, R., and Shanks, J.V. (2011). Re-examination of Metabolic Fluxes in Escherichia coli during Anaerobic Fermentation of Glucose Using 13C Labeling Experiments and 2-dimensional Nuclear Magnetic Resonance (NMR) Spectroscopy. *Biotechnol. Bioprocess. Eng. 16 (3): 419-437*.

3. Murarka, A., Clomburg, J. M., Moran, S., Shanks, J.V., and Gonzalez, R. (2010). Metabolic analysis of wild-type *Escherichia coli* and a pyruvate dehydrogenase complex (PDHC)-deficient derivative reveals the role of PDHC in the fermentative metabolism of glucose. *J. Biol. Chem. 285 (41): 31548-31558.*
4. Blankschien, M. D., Clomburg, J. M., and Gonzalez, R. (2010). Metabolic engineering of *Escherichia coli* for the production of succinate from glycerol. *Metab. Eng. 12 (5): 409-419.*
5. Dellomonaco, C., Rivera, C., Campbell, P., and Gonzalez, R. (2010). Engineered respiro-fermentative metabolism for the production of biofuels and biochemicals from fatty acid-rich feedstocks. *Appl. Environ. Microbiol. 76 (15): 5067-5078*.

(d) Synergistic Activities

- Senior Editor: Journal of Industrial Microbiology and Biotechnology.
- Editorial Board: Applied and Environmental Microbiology; Applied Biochemistry and Biotechnology; Food Biotechnology.
- Program Chair, 2011 Society for Industrial Microbiology Annual Meeting, July 2011, New Orleans, LA.
- Organized and Chaired Sessions on Metabolic Engineering for Biofuels/Biorenewables in AIChE, ACS, and Rice's Institute for Biosystems and Bioengineering meetings.
- Organized and Co-Chaired "Microbial Science and Technology" session, Symposium on Biotechnology for Fuels and Chemicals, San Francisco, CA, 2009.
- Co-Chaired "Rational design in Metabolic Engineering" Session in the Metabolic Engineering V Conference, Lake Tahoe, CA, 2004.
- Participant in DOE workshop on "Biomass to Biofuels" (December 2005), which resulted in a Roadmap for Developing Cleaner Fuels (Report: "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda").
- Ad hoc and panel reviewer for: NSF, USDA, DOE, NIH, and FONDECYT (National Foundation for Science and Technology, Chile).
- Ad hoc reviewer for: Proceedings of the National Academy of Sciences of the USA; Metabolic Engineering; Biotechnology and Bioengineering; Applied and Environmental Microbiology; Applied Microbiology and Biotechnology; Applied Biochemistry and Biotechnology; Genome Research; Molecular Systems Biology; Biophysical Journal; Biotechnology Progress; Food Biotechnology.

(e) Collaborators & Other Affiliations

i. Collaborators

D. Ramkrishna (Purdue University); K.Y. San, M. Wong & P. J. J. Alvarez (Rice University); J. V. Shanks & T. Bobik (Iowa Sate University); C. Altamirano & J. Berrios (Catholic University of Valparaiso, Chile).

ii. Advisor and Postdoctoral Sponsors

***Postdoctoral*:** Prof. Lonnie O. Ingram (Postdoctoral, University of Florida)

***Graduate*:** Prof. Juan A. Asenjo and B. A. Andrews (Ph.D., University of Chile) and Prof. J. C. Gentina (MSc., Catholic University of Valparaiso)

iii. Serving/Served as Advisor (at Rice University and Iowa State University)

- Graduate students: Y. Dharmadi, A. Murarka, A. Gupta, R. San, V. Rigou, J. Clomburg, G. Durnin, C. Dellomonaco, A. Cintolesi, M. Rodriguez-Moya, J. Park, S. Young, S. Kim, S. Dash
- Undergraduate students: T. Pritchard-Meaker, J. Penley, J. Rixen, S. Hothman, M. Preacher, K. Smith, M. Tobelmann, T. Schiling, B. Shite, M. Yu, J. Ross, Z. Yeates, V. Solorzano, K. Wilson, T. Hyunh, J. Miller
- High school students: W. Dewing, O. Quintero, C. Thomas, P. de Guzman, J. Chapman.
- PostDocs: J. Vick, J. Clomburg, E. Miller, M. Blankschien, S. Mazumdar, S. Yazdani

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Iowa State University
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(a) Professional Preparation

University of Kentucky	Chemical Engineering	B.S., 2000
University of California, Los Angeles	Chemical and Biomolecular Engineering	Ph.D., 2006
University of Florida	Florida Center for Renewable Fuels and Chemicals	Postdoctoral Researcher, 2006-2008

(b) Appointments

2010-current	Affiliate member of Bioinformatics & Computational Biology Program, ISU
2008-current	member of Interdepartmental Microbilogy Program, ISU
2008-current	Assistant Professor, Chemical and Biological Engineering, Iowa State University

(c) Publications

i. Five publications most closely related to the proposed project

1. L. R. Jarboe*, Ping Liu, Liam Royce "Engineering inhibitor tolerance for the production of biorenewable fuels and chemicals". *Current Opinion in Chemical Engineering*. 2011 (invited) 1:38-42. DOI 10.1016/j.coche.2011.08.003
2. Yao Fu, Laura R. Jarboe, Julie Dickerson*. "Reconstructing genome-wide regulatory network of *E. coli* using transcriptome data and predicted transcription factor activities". *BMC Bioinformatics*. 2011 12:233.
3. Miller, E.N., L.R. Jarboe, P.C. Turner, P. Pharkya,L.P. Yomano, S.W. York, K.T. Shanmugam, L.O. Ingram. 2009. Furfural Inhibits Growth by Limiting Sulfur Assimilation in Ethanologenic *Escherichia coli* strain LY180. *Applied and Environmental Microbiogy*. 75(19):6132-6141.
4. Miller, E.N., L.R. Jarboe, L.P. Yomano, S.W. York, K.T. Shanmugam, L.O. Ingram. 2009. Silencing of NADPH-dependent Oxidoreductases (*yqhD* and *dkgA*) in Furfural-Resistant Ethanologenic *Escherichia coli*. *Applied and Environmental Microbiology* 75(13):4315-4323.
5. Hyduke, D.R., L.R. Jarboe, L.M. Tran, K.C. Chou and J.C. Liao. 2007. Integrated network analysis identifies nitric oxide response networks and dihydroxyacid dehydratase as a crucial target in *Escherichia coli*. *PNAS* 104(20):8484-8489.

ii. Other significant publications

1. Liu, P., M.L. Soupir, M. Zwonitzer, B. Huss, L. Jarboe***** "Antibiotic Resistance in Agricultural *E. coli* Isolates is Associated with Attachment to Quartz" *Applied and Enironmental Microbiology*. 2011. 77(19):6945-6953.
2. Donovan Layton, A. Ajjarapu, D.W. Choi, L. Jarboe*. "Engineering ethanologenic *Escherichia coli* for levoglucosan utilization". *Bioresource Technology*. 2011 102:8318-8322.
3. L. R. Jarboe***,** Z. Wen, D.W. Choi, R.C. Brown "Hybrid thermochemical processing: fermentation of pyrolysis-derived bio-oil". *Applied Microbiology and Biotechnology*. 2011 (invited) 91(6):1519-1523.

4. Jarboe, L.R. 2010. "YqhD: A broad-substrate range aldehyde reductase with various applications in production of biorenewable fuels and chemical" *Applied Microbiology and Biotechnology.* 89(2):249.
5. L. R. Jarboe, X. Zhang, X. Wang, J.C. Moore, K.T. Shanmugam, L.O. Ingram. 2010. Metabolic engineering for production of biorenewable fuels and chemicals: contributions of synthetic biology *Journal of Biomedicine and Biotechnology.* Article ID 761042

(d) Synergistic Activities

Advisor for the Chemical Engineering Honor Society Omega Chi Epilson at Iowa State University, 2008 - 2011.
Advisor for Iowa State University Engineers Without Borders trips to Belize, March 2010, November 2010
Designed and implemented a fermentation module for CBiRC RET program, summer 2009
Research experience for Undergraduates, Young Engineers and K-12 Teachers:
- ISU students (24), REU students (5), Young Engineers (2), RET (1)
Session (co-)chair for Society for Industrial Microbiology (2009, 2010, 2011, 2012), American Institute of Chemical Engineers (2009, 2010, 2011, 2012)

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Srinivas Aluru, Iowa State University
Robert Brown, Iowa State University
Nancy DaSilva, University of California, Irvine
Julie Dickerson, Iowa State University
Ramon Gonzalez, Rice University
Tammy Grabar, BioEnergy
Cathy Logue, Iowa State University
Elliot Miller, University of Florida
David Nielsen, Arizona State University
Lisa Nolan, Iowa State University
D. Raj Raman, Iowa State University
Derrick Rollins, Iowa State University
Ka-Yiu San, Rice University
Suzanne Sandmeyer, Univ. of California, Irvine
Jackie Shanks, Iowa State University
K. T. Shanmugam, University of Florida
Michelle Soupir, Iowa State University
Michael Thompson, Iowa State University
Peter C. Tuner, University of Florida
Larry Wackett, University of Minnesota
Xhiyou Wen, Iowa State University
Xueli Zhang, University of Florida

ii. Graduate Advisors and Postdoctoral Sponsors

James C. Liao	University of California, Los Angeles
Lonnie O. Ingram	University of Florida

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Tao Jin	Iowa State University, Chemical and Biological Engineering
Ping Liu	Iowa State University, Microbiology
Liam Royce	Iowa State University, Chemical and Biological Engineering
Kumar B. Kautharapu	Iowa State University, Chemical and Biological Engineering
Martha Zwonitzer	Environmental Sciences

6 = Total number of graduate students advised and postdocs sponsored.

PETER L. KEELING
Director, Industrial Collaboration & Innovation
NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
Iowa State University, 1140 Biorenewables Research Laboratory, Ames, IA 50011-3270
(515) 294-4093 / (515) 294-1269 (fax) / pkeeling@iastate.edu

(a) Professional Preparation

Hertfordshire University, UK	Applied Biology	B.S., 1972-1976
Council Nat. Academic Awards, UK	Biochemistry and Toxicology	Ph.D., 1976-1981
ICI/Zeneca Seeds, Cheshire, UK	Biochemistry	Postdoc., 1981-1984

(b) Appointments

2009 – present	Director, Industrial Collaboration & Innovation Program, CBiRC, Iowa State University, Ames, IA
2007 – present	Founder and Director, EnaGen LLC, Ames, IA and Research Professor, Iowa State University, Ames, IA.
2000 – 2007	Unit R&D Director, ExSeed Genetics Research, BASF Plant Science, 2901 South Loop Drive, Bldg3 Suite 3800, Ames, IA.
1994 – 2000	Founder and Research Director, ExSeed Genetics L.L.C., Food Science Building, Iowa State University, Ames, IA.
1988 – 1994	Group Manager, ICI/Zeneca Seeds, Biochemistry, Cytogenetics and Physiology Group, Slater, IA.
1981 – 1988	Project Leader, ICI/Zeneca Seeds, Grain Filling Project, Bioscience Research Laboratory, Runcorn, Cheshire, UK
1976 – 1981	Scientist, ICI Central Toxicology Laboratory, Pulmonary Toxicology Group, Biochemical Mechanisms Section, Alderley Park, Cheshire, UK

(c) Publications

i. Five publications most closely related to the proposed project

1. Keeling, P.L. and Myers, A.M. Biochemistry and Genetics of Starch Synthesis, *Annual Review of Food Science and Technology, Annual Reviews.* **1** 271-303 (2010)
2. Hennen-Bierwagen TA, Lin Q, Grimaud F, Planchot V, Keeling PL, James MG, Myers AM. Proteins from Multiple Metabolic Pathways Associate with Starch Biosynthetic Enzymes in High Molecular Weight Complexes: A Model for Regulation of Carbon Allocation in Maize Amyloplasts. *Plant Physiology*. **146** 1541-1559 (2009).
3. Klucinec J.D. and Keeling P.L. Genetic modifications of plant starches for food applications. *In: Functional Foods & Biotechnology* (Vol 165, Food Science and Technology). Ed: Shetty, Paliyath, Pometto and Levin. Taylor & Francis, New York. (2006).
4. Tziotis A, Seetharaman K, Klucinec JD, Keeling P, White PJ. Functional properties of starch from normal and mutant corn genotypes. *Carbohydrate Polymers*: **61**: 238-247. (2005).
5. Gao Z., Guan H. and Keeling P.L. Involvement of lysine-193 of the conserved "KTGG" motif in the catalysis of maize starch synthase IIa. *Annals Biochemistry and Biophysics*, **427**: 1-7. (2004).

ii. Five other significant publications

1. Imparl-Radosevich J.M., Gameon, J.R., McKean, A., Wetterberg. D., Keeling P.L. and Guan H. Understanding catalytic properties and functions of maize starch synthase enzymes. *Journal of Applied Glycoscience*, **50**: 177-182. (2003).
2. Commuri P.D. and Keeling P.L. Chain-length specificities of maize starch synthase I enzyme: studies of glucan affinity and catalytic properties. *The Plant Journal* **25**(5): 475-486. (2001).
3. Miller S.A., Artuso A., Avery D, Beachy R.N., Day P.R., Fennema O.R., Hardy R., Keeling P.L., Klaenhammer T.R., McGloughlin M., Vidaver A.K. Benefits and concerns associated with recombinant DNA biotechnology-derived foods. Benefits and Concerns Expert Panel. *Institute of Food Technologists. Food-Technology*: **54** (10) 61-80. (2000).
4. Keeling P.L. From enzyme activity to flux control: a quest to understand starch deposition in developing cereal grains. *In: Plant Carbohydrate Biochemistry.* Eds. JA Bryant, MM Burrell and NJ Kruger. BIOS Scientific Publishers, Oxford UK. (1999).
5. Guan H. and Keeling P.L. Starch Biosynthesis: Understanding the functions and interactions of multiple isozymes of starch synthase and branching enzyme. *Trends in Glycoscience and Glycotechnology* **10**: 307-319. (1998)

(d) Synergistic Activities

2009–present	Multiple oral presentations at Infocast BioBased Summits, Bio World Congress meetings and Food Science Meetings.
2007–present	Multiple grant proposal submissions to USDA/NSF as PI/Co-PI with Drs Alan Myers and Martha James, ISU, Biochem, Biophys & Molec Biology Dept, Ames, IA.
1994–present	Member of the ISU Interdepartmental Major in Plant Biology and POS Committee PhD and Masters Students as Affiliate Professor, Dept. Agronomy, ISU, Ames, IA.
1994–2009	Board Member, Starch Round Table, American Association of Cereal Chemists.
Jan. 2009	Presentation on "Managing Startup Growth and Coping with Changes following Acquisition". Iowa Entrepreneurship Forum, Iowa State University Research Park.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Bryant, Jonathan	BASF Corporation, RTP, NC
Denyer, Kay	The John Innes Center, UK
Grimaud, Florent	Institut National de la Recherche Agronomique, Nantes, France
Guan, Hanping	BASF Corporation, RTP, NC
James, Martha	Iowa State University, Ames, IA
Klucinec, Jeff	BASF Corporation, Ames, IA
Logemann, Juergen	BASF Corporation, Limburgerhof, Germany
Myers, Alan	Iowa State University, Ames, IA
Seetharaman, Kousheik	University of Guelph, Canada
White, Pam	Iowa State University, Ames, Iowa

ii. Graduate and Postdoctoral Advisors

Aldridge, Norman	MRC Toxicology Unit, Carshalton, Surrey, UK
Bridges, Ian	Syngenta, United Kingdom
Smith, Lewis	Astra Zeneca, United Kingdom

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Miller, Rachel	Iowa State University, Ames, Iowa

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University Professor, Chemistry Department
Iowa State University
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(a) Professional Preparation

University of Rochester	Chemistry	B.S., 1972
Columbia University (G. Stork)	Chemistry	Ph.D., 1976

(b) Appointments

2004 – present	University Professor, Chemistry, Iowa State University
1993 – 1999	Chair, Chemistry Department, Iowa State University
1986 – 2004	Professor, Chemistry, Iowa State University
1981 – 1986	Associate Professor, Chemistry, Iowa State University
1976 – 1981	Assistant Professor, Chemistry, Iowa State University

(b) Publications

i. Five publications most closely related to the proposed project

1. G. A. Kraus, S. Riley, T. Cordes "Aromatics from Pyrones: Para-Substituted Alkyl Benzoates from Alkenes and Coumalic Acid and Methyl Coumalate" *Green Chem*. **2011**, 2734-2736.
2. G. A. Kraus, I. Jeon, J. Mengwasser, T. Windus "Metalation Selectivity of 4,6-Dibromoresorcinol Dimethyl Ether", *Synlett*, **2010**, 1955.
3. G. A. Kraus, V. Gupta "A New Synthetic Strategy for the Synthesis of Bioactive Stilbene Dimers. A Direct Synthesis of Amurensin H", *Tetrahedron Letters*, **2009**, *50*, 7180-7183.
4. G. A. Kraus, H. Guo "A Flexible Synthesis of 2,3-Disubstituted Indoles fromAminobenzyl Phosphoniun Salts. A Direct Synthesis of Rutaecarpine" *J. Org. Chem*. **2009**, *74*, 5337.
5. G. A. Kraus, H. Guo "One-Pot Synthesis of 2-Substituted Indoles from 2-Aminobenzyl Phosphonium Salts. A Formal Total Synthesis of Arcyriacyanin A", *Org. Lett*, **2008**, *10*, 3061-3063.

ii. Five other significant publications

1. S. Bose, A. Wijeratne, A. Thite, G. A. Kraus, D. Armstrong, J. Petrich, "Influence of Chiral Ionic Liquids on Stereoselective Fluorescence Quenching by Photoinduced Electron Transfer in a Naproxen Dyad" *J. Phys. Chem, B*, **2009**, *113*, 10825.
2. G. A. Kraus, A. Kempema "A Direct Synthesis of Racemic Tetragalloyl Apiitol", *Synthesis*, **2010**, 389.
3. G. A. Kraus, T. Wu "A Three-Component Reaction Between Benzynes, the Enolate of Acetaldehyde, and Unsaturated Esters and Dihydroisoquinolines", *Tetrahedron*, **2010**, *66*, 569-572.
4. G. A. Kraus, V. Gupta, M. Mokhtarian, S. Mehanovic and M. Nilsen-Hamilton "New Effective Inhibitors of the Abelson Kinase", *Bioorg. Med. Chem*., **2010**, *18(17)*, 6316-21.
5. G. A. Kraus, J. Mengwasser, W. Maury "Synthesis of Chroman Aldehydes that Inhibit HIV" *Bioorg. Med. Chem. Lett*. **2011**, *21*, 1399-1401.

(c) Synergistic Activities

Assistant Director for bio-related activities in the Ames Laboratory, 2004-2007
Director of the Institute for Physical Research and Technology
PI of successful DOE grant "Development of A Biobased Graduate Minor"
College of Liberal Arts and Sciences Award for Excellence in Research/Artistic Creativity (2001)
Co-PI of NIH Center grant to examine botanical dietary supplements

(d) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Dr. Victor Lin, Iowa State University
Dr. Marit Nilsen-Hamilton, Iowa State University
Dr. Pranav Shrotriya, Iowa State University
Dr. Greg Phillips, Iowa State University
Dr. John Verkade, Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors

Gilbert Stork, Columbia University

ii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Sean Riley (Ph.D. 2011, Iowa State University), John Mengwasser (Ph.D. 2010, Shell), Vinayak Gupta (Ph.D. 2010, Scripps Florida), Insik Jeon (Ph.D. – 2007, Columbia University), Sarathy Kesavan (Ph.D. – 2003, Boston University), Ikyon Kim (Ph.D. – 2002, Wisconsin), Junwon Kim (Ph.D. – 2003, Johns Hopkins), Yeung-Ho Seo (Ph.D. – 2006, Michigan), Jinqiang Wei (Ph.D. – 2006, Broad Institute), G. Dai (M.S. – 1999, Glaxo Smith Kline), H. Ogutu (M.S. – 2001, Bayer), Dr. Sudipta Roy (Postdoc 2006 – 2007), Dr. P.K. Choudhury (Postdoc 1998 – 2002), Dr. Wenge Cui (Postdoc 1999 – 2000).

90 = Total number of graduate students advised and postdocs sponsored.

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(a) Professional Preparation

University of California, Davis	Chemistry	B.S., 1967
Purdue University	Chemistry	Ph.D., 1972
Harvard University	Chemistry	Postdoc, 1971 – 1972

(b) Appointments

2007 – present	Distinguished Professor, Chemistry, Iowa State University
1999 – present	University Professor, Chemistry, Iowa State University
1985 - 1999	Professor, Chemistry, Iowa State University
1985 – 1985	Visiting Associate Professor, University of Hawaii
1978 – 1985	Associate Professor, Chemistry, Iowa State University
1974 – 1978	Assistant Professor, Chemistry, Iowa State University
1972 – 1974	Instructor, Chemistry, Iowa State University

(c) Selected Recent Publications (out of ~370 total publications)

1. Zhang, X.; Yao, T.; Campo, M. A.; Larock, R. C. "Synthesis of Substituted Quinolines by the Electrophilic Cyclization of *N*-(2-Alkynyl)anilines," *Tetrahedron* **2010**, *66*, 1177-1187.
2. Xia, Y.; Lu, Y.; Larock, R. C. "Ring-Opening Metathesis Polymerization (ROMP) of Norbornenyl-Functionalized Fatty Alcohols," *Polymer* **2010**, *51*, 53-61.
3. Rogness, D. C.; Larock, R. C. "Synthesis of Acridines by the [4+2] Annulation of Arynes and 2-Aminoaryl Ketones," *J. Org. Chem.* **2010**, *75*, 2289-2295.
4. Shi, F.; Larock, R. C. "Remote C-H Activation via Through-Space Palladium Migration," a chapter in Topics in Current Chemistry, Springer-Verlag: Berlin-Heidelberg-New York, **2010**, Vol. 292, pp. 123-164.
5. Mehta, S. Larock, R. C. "Iodine/Palladium Approaches to the Synthesis of Polyheterocyclic Compounds," *J. Org. Chem.* **2010**, *75*, 1652-1658.
6. Cho, C.-H.; Neuenswander, B.; Larock, R. C. "A Diverse Methyl Sulfone-Containing Benzo[*b*]thiophene Library via Iodocyclization and Palladium-Catalyzed Coupling," *J. Comb. Chem.* **2010**, *12*, 278-285.
7. Dubrovskiy, A. V.; Larock, R. C. "Synthesis of Benzisoxazoles by the [3 + 2] Cycloaddition of *in situ* Generated Nitrile Oxides and Arynes," *Org. Lett.* **2010**, *12*, 1180-1183.
8. Xia, Y.; Larock, R. C. "Castor Oil-based Thermosets with Varied Crosslink Densities Prepared by Ring-opening Metathesis Polymerization (ROMP)," *Polymer* **2010**, *51*, 2508-2514.
9. Pfister, D. P.; Larock, R. C. "Thermophysical Properties of Conjugated Soybean Oil/Corn Stover Biocomposites," *Bioresour. Technol.* **2010**, *101*, 6200-6206.
10. Rozhkov, R. V.; Larock, R. C. "Synthesis of Dihydrobenzofurans via Palladium-Catalyzed Annulation of 1,3-Dienes by o-Iodoaryl Acetates," *J. Org. Chem.* **2010**, *75*, 4131-4134.
11. Pfister, D. P.; Larock, R. C. "Green Composites from a Conjugated Linseed Oil-Based Resin and Wheat Straw," *Composites Part A* **2010**, *42*, 1279-1288.

12. Lu, Y.; Larock, R. C. "Aqueous Cationic Polyurethane Dispersions from Vegetable Oils", *ChemSusChem* **2010**, *3*, 329-333.
13. Lu, Y.; Larock, R. C. "Soybean Oil-Based, Aqueous Cationic Polyurethane Dispersions: Synthesis and Properties," *Prog. Org. Coat.* **2010**, *69*, 31-37.
14. Dubrovskiy, A. V.; Larock, R. C. "Intermolecular C-O Addition of Carboxylic Acids to Arynes," *Org. Lett.* **2010**, *12*, 3117-3119.
15. Cho, C.-H.; Larock, R. C. "A Convenient Synthetic Route to Furan Esters and Lactones by Palladium-Catalyzed Carboalkoxylation or Cyclocarbonylation of Hydroxyl-Substituted 3-Iodofurans," *Tetrahedron Lett.* **2010**, *51*, 3417-3421.
16. Xia, Y.; Larock, R. C. "Vegetable Oil-based Polymeric Materials: Synthesis, Properties, and Applications," *Green Chemistry* **2010**, *12*, 1893-1909.
17. Wu, C.; Fang, Y.; Larock, R. C.; Shi, F. "Synthesis of 2H-Indazoles by the [3+2] Cycloaddition of Arynes and Sydnones," *Org. Lett.* **2010**, *12*, 2234-2237.
18. Shi, F.; Larock, R. C. "Synthesis of Substituted Indazoles via [3+2] Cycloaddition of Benzyne and Diazo Compounds," *Org. Syn.* **2010**, *87*, 95-103.
19. Kivrak, A.; Larock, R. C. "Synthesis of Dihydrobenzisoxazoles by the [3+2] Cycloaddition of Arynes and Oxaziridines," *J. Org. Chem.* **2010**, *75*, 7381-7387.

(d) Synergistic Activities

Author of the best-selling book "Comprehensive Organic Transformations," 2nd ed., "Organomercury Compounds in Organic Synthesis" and "Solvomercuration/Demercuration Reactions in Organic Synthesis."

Editorial Advisory Boards, Journal of Biobased Materials and Bioenergy, and the Open Agriculture Journal; Consulting - Cargill; Scientific Advisory Board – Segetis and the Brazilian Meeting on Organic Synthesis.

Awards: Merck Academic Development Awards (1997 and 1998); 2003 ACS Edward Leete Award; 2004 Paul Rylander Award of the Organic Reactions Catalysis Society; 2004 ACS Arthur C. Cope Senior Scholar Award.

(e) Collaborators & Other Affiliations

Collaborators (past 48 months): Michael Kessler (ISU)

Graduate and Postdoctoral Advisors: Herbert C. Brown - deceased (Purdue University, Ph.D.); Elias J. Corey (Harvard; Postdoc)

Recent Students (past 48 months): Jian Zhao (8/01-2/07), Jesse Waldo (8/02-8/08), Tanay Kesharwani (8/02-8/08), Ziwei Wu Just (8/03-8/07), Phillip Henna (8/03-8/08), Shilpa Worlikar (8/02-8/08), Marlen Valverde (8/03-8/09), Saurabh Mehta (1/04-11/09), Arif Kivrak (7/08-1/09), Chun Lu (8/04-6/10), Daniel Pfister (8/05-12/10).

Recent Postdoctoral Fellows (past 48 months): Sujata Roy (5/07-5/08), Sudipta Roy (10/07-5/08), Niangui Wang (9/07-8/08), Akhilesh Verma (6/07-8/08), Raffaella Mancuso (7/08-12/09), Dai-Il Jung (6/08-2/09), Feng Shi (2/07-5/09), Yu Chen (1/07-8/09), Chul-Hee Cho, (2/07-11/09), Dr. Yongshang Lu (4/05-2/10).

Current Students: Donald Rogness (8/05), Yuesi Fang (8/06), Rafael Quirino (8/06), Ying Xia (8/07), Anton Dubrovskiy (8/07), Nataliya Markina (8/07), Thomas Garrison (6/09).

Total number of graduate students advised and postdocs sponsored = 116.

ADAH LESHEM
Pre-College Education Program Director
NSF Engineering Research Center for Biorenewable Chemicals
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(a) Professional Preparation

King's College, University of London, UK	Environmental Science	B.Sc. (w/ Honors), 1980
University of Cambridge, UK	Applied Biology	M. Phil., 1981
Tel-Aviv University, Israel	Environment Physiology	Ph.D., 1989

(b) Appointments

2003 – present	Program Director, Pre-College Education Outreach, Iowa State University
2000 – 2002	Adjunct Assistant Professor, Department of Zoology and Genetics, Iowa State University
1997 – 2002	Program Coordinator, International Institute of Theoretical and Applied Physics, Iowa State University
1995 – 1997	Advising Coordinator, Biology Program, Iowa State University
1986 – 1992	Temporary Assistant Professor, Department of Zoology and Genetics, Iowa State University

(c) Publications

i. Five publications most closely related to the proposed project

N/A

ii. Five other significant publications

N/A

(d) Synergistic Activities

1. Director of Pre-College Education, NSF Engineering Research Center for Biorenewable Chemicals. Develop, implement and oversee various professional development programs for science K-12 teachers including and RET program, a 4-week summer institute for middle school teachers, and a one-week training program for elementary school teachers focusing on plants in society. (http://www.cbirc.iastate.edu/precollege.asp)
2. Director, Research Opportunities in Molecular Biology, Biotechnology and Genomics, a summer research experience program for middle/high school biology teachers (RET) that includes molecular biotechnology and genomics theory and technique training, curriculum and instruction development and a six week research component. (http://www.plantgenomeoutreach.eeob.iastate.edu/)
3. Director, Symbi, Iowa's GK12 program. (http://www.gk12.iastate.edu/)
4. Director, "Partnerships for Research Education in Plants" in Iowa. This program provides genuine research experiences to over 300 high school students in Iowa as well as teachers, while helping scientists to discover the function of previously uncharacterized plant genes. (http://www.prep.biotech.vt.edu/index.html)
5. Director, "Partnerships for Science and Engineering Education at Iowa State University" which provides high school students with a semester long, extracurricular, research experience under the

mentorship of science and engineering faculty at Iowa State University. (http://www.plantgenomeoutreach.eeob.iastate.edu/HSS.htm)

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Volker Brendel, Iowa State University
Anne Bronikowski, Iowa State University
Dawn Del Carlo, University of Northern Iowa
Drena Dobbs, Iowa State University
Erin Dolan, Virgina Polytechnic Institute and State University
Basil Nikolau, Iowa State University
Thomas Peterson, Iowa State University
Raj Raman, Iowa State University
Laurel Southard, Cornell University
Martin Spalding, Iowa State University
Brent Shanks, Iowa State University
Mack Shelley, Iowa State University
Jay Staker, Iowa State University
Dan Voytas, University of Minnesota
Jeff Weld, University of Northern Iowa
Jonathan Wendel, Iowa State University
Roger Wise, Iowa State University
Steve Whitham, Iowa State University
Eve Wurtele, Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors

Ralph Ackerman, Iowa State University
Amos Ar, Tel-Aviv University, Israel

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

N/A

COSTAS D. MARANAS
Donald B. Broughton Professor
Department of Chemical Engineering
The Pennsylvania State University
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(a) Professional Preparation

1990-1995	Princeton University, Princeton, New Jersey Ph.D. in Chemical Engineering, May 1995 M.A. in Chemical Engineering, June 1992
1985-1990	Aristotle University, Thessaloniki, Greece Diploma in Chemical Engineering, June 1990

(b) Appointments

Jun. 2004-present	The Pennsylvania State University, Department of Chemical Engineering Professor
June 2005-present	Donald B. Broughton Professor in Chemical Engineering
May 2005-present	Director of Graduate Student Recruiting for the Bioinformatics & Genomics Option
Jan. 2004-present	Faculty Affiliate of the Center for Supply Chain Research
Sep. 2001-present	Member of Faculty of the Intercollege Graduate Degree in Integrative Biosciences (Bioinformatics & Genomics Option)
Jun. 2001-June 2004	The Pennsylvania State University, Department of Chemical Engineering Associate Professor
Sep. 1997-present	Member of Faculty of the Operations Research Program
Sep. 1995-Jun. 2001	The Pennsylvania State University, Department of Chemical Engineering Assistant Professor
Sept. 1990.-May 1995.	Princeton University, Dept. of Chemical Engineering Research Assistant
Feb.-Jul. 1990	Chemical Process Research Institute (CPERI), Greece Research Assistant

(c) Publications

i. Five publications most closely related to the proposed project

1. Ranganathan, S. and C.D. Maranas (2010), "Microbial 1-butanol production: Identification of non-native production routes and *in silico* engineering interventions," *Biotechnology Journal*, Vol. 5, 716-725.
2. Ranganathan, S., P.F. Suthers and C.D. Maranas (2010), "OptForce: An Optimization Procedure for Identifying All Genetic Manipulations Leading to Targeted Overproductions," *PLoS Comput. Biol*., Vol. 6, No. 4, e1000744.
3. AbuOun, M., P.F. Suthers, G.I. Jones, B.R. Carter, M.P. Saunders, C.D. Maranas, M.J. Woodward and M.F. Anjum (2009), "Genome Scale Reconstruction of a Salmonella Metabolic Model," *The Journal of Biological Chemistry, Vol. 284,* No. 43, 29480-29488.
4. Zomorrodi, A., P.F. Suthers and C.D. Maranas (2009), "Genome-scale Gene/Reaction Essentiality and Synthetic Lethality Analysis," *Molecular Systems Biology, Vol. 5*, No. 301, Aug. 18.

5. Kumar, V.S. and C.D. Maranas (2009), "GrowMatch: An automated method for reconciling in vivo/in silico growth predictions," *PLoS Comput. Biol., Vol. 5*, No. 3, e1000308.

ii. Five other significant publications

1. Suthers, P.F., M.S. Dasika, V.S. Kumar, G. Denisov, J.I. Glass, and C.D. Maranas (2009), "A genome-scale metabolic reconstruction of Mycoplasma genitalium iPS189," *PLoS Comput. Biol., Vol. 5*, No. 2, e1000285.
2. Cheng, Y.J., P.F. Suthers, and C.D. Maranas (2008), "Identification of Optimal Measurement Sets for Complete Flux Elucidation in MFA Experiments," *Biotechnology & Bioengineering, Vol. 100*, No. 6, 1039-1049.
3. Satish Kumar V., M.S. Dasika, and C.D. Maranas (2007), "Optimization based automated curation of metabolic reconstructions," *BMC Bioinformatics, Vol. 8,* 212.
4. Suthers, P.F., A.P. Burgard, M.S. Dasika, F. Nowroozi, S. Van Dien, J.D. Keasling, and C.D. Maranas (2007), "Metabolic Flux Elucidation for Genome-Scale Models Using ^{13}C Labeled Isotopes," *Metabolic Engineering, Vol. 9*, 387-405.
5. Burgard, A., P. Pharkya and C.D. Maranas (2003), "OptKnock: A Bilevel Programming Framework for Identifying Gene Knock-Out Strategies for Microbial Strain Optimization," *Biotechnology & Bioengineering, Vol.84*, No. 6, 647-657.

(d) Synergistic Activities

1. Chair of Biochemical and Biomolecular Engineering XVII – *Emerging Frontiers* conference, Seattle WA, June 19-24, 2011.
2. Participant in Extreme Computing Opportunities in Biology workshop, Chicago IL, July 16, 2009.
3. Participant in the BER-NERSC Large Scale Computing and Storage Requirements for Biological and Environmental Research workshop, Rockville MD, May 7-8, 2009.
4. Fellow of the American Institute for Medical and Biological Engineering (Feb. 2008-present)
5. Editorial Board Member: PLoS Computational Biology, Biotechnology Journal, Biophysical Journal, and Metabolic Engineering

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors

Collaborators at Penn State include Profs. Reka Albert, Antonios Armaou, Stephen J. Benkovic, Howard Salis, Patrick Cirino, Michael Janik, Christine Keating, Gary Clawson and Tom Richard.

Collaborators and co-Editors outside Penn State include Prof. Maciek Antoniewicz (U. Delaware), Francois Banyex (U. of Washington), Beth Junger (Merck), Peter Karp (BioCyc), Anjum Muna (Veterinary Lab. Agency, UK), Himadri Pakrashi (Wash. U.), Lou Sherman (Purdue U.), Terry Papoutsakis (U. of Delaware), Mattheos Koffas (U. of Buffalo), James Liao (UCLA), Greg Stephanopoulos (MIT), Jay Keasling (UCB), Cathy H. Wu (U. of Delaware), Diego di Bernardo (U. of Naples), and Jason Papin (U. of Virginia).

ii. Graduate and Postdoctoral Advisors

Prof. Christodoulos A. Floudas, Princeton University (1990-1995)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor

Profs. K. Camarda (U. Kansas), Evgeni Nikolaev (Strayer Univ.) and Drs. G. Moore (Xencor, Inc.), S. Vaidyaraman (Eli Lilly), A. Burgard (Genomatica, Inc.), P. Pharkya (Genomatica, Inc.), A. Lehmann (Fox Chase Cancer Center), Mike Rodgers (DuPont), A. Gupta (SCA), M. Saraf (City Group), M. Dasika (Amyris), Hossein Fazelinia (Fox Chase Cancer Center), YoungJung Chang (CMU), Vinay Satish Kumar (JBEI), Francisco Vital-Lopez (BHSAI), and Sridhar Ranganathan (Life Technologies).

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(a) Professional Preparation

Iowa State University	Economics	B.S., 1989
Iowa State University	Fisheries & Wildlife Biology	B.S., 1992

(b) Appointments

2008 – present	Assistant Director (Administrative Director), NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2006 – 2008	Business Manager, Bioeconomy Institute, Iowa State University
2001 – 2006	Program Coordinator, Pre- and Post-Award Administration, Office of Biorenewables Programs, Iowa State University
1996 – 2001	Program Assistant, Center for Sustainable and Environmental Technologies, Iowa State University
1993 – 1996	Program Assistant, Center for Coal and the Environment, Iowa State University, and Fossil Energy Program, Ames Laboratory, US DOE

(c) Publications

N/A

(d) Synergistic Activities

2010-2011, Chair, Equity, Diversity and Student-Athlete Well-Being Subcommittee, NCAA Certification Campaign, Iowa State University
2007-2011, Chair, Equity Committee, Athletics Council, Iowa State University
2005-2011, Member, Athletics Council, Iowa State University

(e) Collaborators and Other Affiliations

Collaborators and Co-Editors

N/A

Graduate Advisors and Postdoctoral Sponsors

N/A

Thesis Advisor and Postgraduate-Scholar Sponsor

N/A

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(a) Professional Preparation

Michigan State University	Chemical Engineering	B.S., 1986
University of Delaware	Chemical Engineering	Ph.D., 1992
Schuit Institute of Catalysis	Chemical Engineering	Postdoc, 1992 - 1993

(b) Appointments

2005 – present	Alice M. & Guy A. Wilson Professor of Engineering, University of Virginia
2003 – present	Professor of Chemical Engineering, University of Virginia
2003 – present	Professor of Chemistry, University of Virginia
2000 – 2003	Associate Professor of Chemical Engineering, University of Virginia
2002	Technical Advisory Board for Heterogeneous Metathesis Catalysis, Dow Chemical Company
2001 – present	Editorial Board, Catalysis Communications
2001 – present	Board of Visitors, Department of Chemical Engineering, Michigan State Univ.
1995 – 1999	Assistant Professor of Chemical Engineering
1993 – 1995	*Visiting Research Scientist, DuPont Central Research and Development,* Corporate Catalysis Center, Experimental Station, Wilmington, DE.
1993 – 1995	Visiting Research Engineer, Department of Chemical Engineering, University of Delaware, Newark DE.

(c) Publications

i. Five publications most closely related to the proposed project

1. Neurock, M., M. Janik, and A. Wieckowski, A First Principles Comparison of the Mechanism and Site Requirements for the Electrocatalytic Oxidation of Methanol and Formic Acid over Pt, *Farad. Trans*., accepted, 2008.
2. Taylor, C.D., R.G. Kelly, and M. Neurock, First-principles predictions of equilibrium potentials for the electrochemical activation of water by a series of transition and precious metals, *J. Electro. Soc.*, **154** (12): F217-F221 2007.
3. Janik, M.J., C.D. Taylor, and M. Neurock, "First Principles Analysis of the Electrocatalytic Oxidation of Methanol and Carbon Monoxide", *Topics in Catal.*, **46**, 306-319, 2007.
4. Janik, M.J., and M. Neurock, "A first principles analysis of the electro-oxidation of CO over Pt (111)", *Electrochemica Acta*, **52**, 18, 2007, Pages 5517-5528.
5. Taylor, C.D., R.G. Kelly, and M. Neurock, "First-Principles Analysis of the Electronic Interactions between Hydroxyl and Copper at the Electrochemical Interface", *J. Electroanal. Chem.*, **607** (1-2): 167-174, 2007.

ii. Other significant publications

1. Taylor, C.D., R.G. Kelly, and M. Neurock," Theoretical analysis of the nature of hydrogen at the electrochemical interface between water and a Ni(111) single-crystal electrode", *J. Electrochem. Soc.*, **154** (3): F55-F64 2007.
2. Rossmeisl, J., J. Norskov, C. D. Taylor, M.J. Janik, and M. Neurock, "Calculated phase diagrams for the electrochemical oxidation and reduction of water over Pt(111)", *J. Phys. Chem. B*, **110**, 43, 2183-21839, 2006.

3. Filhol, J. S., and M. Neurock, "First-Principles Elucidation of the Electrochemical Activation of Water over Pd", *Angew. Chemie Internation (Frontispiece)*, **45**, 3, 402 – 406 (2005).
4. Taylor, C.D., S. A. Wasileski, J. Fanjoy, J.S. Filhol, M. Neurock, First Principles Reaction Modeling of the Electrochemical Interface: Consideration and Calculation of a Tunable Surface Potential from Atomic and Electronic Structure", *Phys. Rev. B*, **73**, 165402, 2006.
5. Cao, D., Lu, G,-Q., A. Wieckowski, S.A. Wasileski, and M. Neurock, Mechanisms of Methanol Decomposition on Platinum: A Combined Experimental and Ab Initio Approach, *J. Phys. Chem. B.*, **109** (23): 11622-11633, 2005.

(d) Synergistic Activities

Editor for the Journal of Catalysis
Panel Member: International Study by the World Technology Evaluation Center and the National Science Foundation, "Catalysis by Nanostructured Materials"
Advisory Board, Institute for Interfacial Catalysis, Pacific Northwest Lab., 2003- present
Director Catalysis and Reaction Engineering (Division 20), AICHE, (November 2003-2007)
Liaison of the South Eastern Catalysis Soc. to the North American Catalysis Soc. (2003-2006)

(e) Collaborators & Other Affiliations

i. Collaborators (last 48 months)

A. Anderson, CWRU
L. Broadbelt, NWU
G. Ceder, MIT
R. J. Davis, UVa
J. Elam, Argonne
M. Flytzani, Tufts
R. Gorte, Penn
G. Haller, Yale
H. Kung, NWU
M. Kung, NWU
T. Marks, NWU
N. Marzari, MIT
J. Nørskov, Tech. Univ., Denmark
L. Pfefferle, Yale
G. Scuseria, Rice
P. Stair, NWU
W. Tysoe, UWM
S. Vajda, Argonne
R. van Santen, Eindhoven Univ. Tech.
I. Wachs, Lehigh
A.Wieckoski, UICU
M. Wong, Rice
T. Zawodzinski, CWRU

ii. Graduate and Postdoctoral Advisors

Michael T. Klein (Rutgers Univ.), Rutger A. van Saten (Eindhoven Univ. of Tech., Netherlands)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Past Students:
Pallassana Venkataraman, Eric Hansen, Sanket Desai (Ph.D., Exxon Mobil), Hongmei Wen (Ph.D., United Tech.), Priyam Sheth (Ph.D., Shell Chemical Co.), Michael Janic (Ph.D., PSU), Christopher Taylor (Ph.D., LANL), Cheng Ying Lee, Neeti Kapur, Vamsi Vadhri. Postdocs: Dr. R. Meyer, Dr. Steven Mitchell, Dr. Sally Wasileski, Dr. Qingfeng Ge, Dr. Donghai Mei, Dr. Michael Palmer, Dr. Jean Sebastian Filhol, Dr. David Wathall, Dr. Yu Cai, Dr. Michael Janik, Dr. Christopher Taylor, Dr. Corneliu Buda, Dr. Jincheng Du
Current Students:
E. Caruana, N. Kapur, N. Sinha, C. Lee, C. Plaisance, V. Vadhri, C. Buda, J. Du, Y. Cai

JOSEPH P. NOEL
Professor-Director & Investigator, Jack H. Skirball Center for Chemical Biology & Proteomics
The Salk Institute for Biological Studies & Howard Hughes Medical Institute
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(a) Professional Preparation

University of Pittsburgh at Johnstown	Natural Sciences/Chemistry	B.S. – 1985
The Ohio State University	Chemistry/Biochemistry	Ph.D. – 1990
Yale University	Structural Biology	1990-1994

(b) Appointments

2005-present	Director, Jack H. Skirball Center for Chemical Biology and Proteomics, Salk Institute
2005-present	Professor, Jack H. Skirball Center for Chemical Biology and Proteomics, Salk Institute
2005-present	Investigator, Howard Hughes Medical Institute
2003-present	Adjunct Professor, Div. Biology and Dept. Chem. & Biochem., Univ. California, San Diego
2002-2004	Professor, Structural Biology Laboratory, Salk Institute
2000-2002	Adjunct Associate Professor, Depts. Biology and Chem. & Biochem., Univ. California, San Diego
1999-2002	Associate Professor, Structural Biology Laboratory, Salk Institute
1997-2000	Adjunct Assistant Professor, Dept. Biology, Univ. California, San Diego
1994-2000	Adjunct Assistant Professor, Dept. Chem. & Biochem., Univ. California, San Diego
1994-1999	Assistant Professor, Structural Biology Laboratory, Salk Institute
1990-1994	NSF Chemistry Postdoctoral Fellow, Professor Paul B. Sigler, Yale Univ.
1985-1990	Graduate Student, Dept. Chem., Ohio State Univ.

(c) Publications (total publications 131 - h-index = 46)

(i) Five Most Related to the Proposed Project

1. Zheng, Z.*, Guo, Y.*, Novak, O., Ljung, K., Noel, J.P.* and Chory, J.* (2012) Coordination of Auxin and Ethylene Biosynthesis Modulates Plant Shade Avoidance Respones. *Nature* (submitted) (*equal contributions / co-corresponding authors).
2. Auldridge, M.E., Austin, M.B., Ramsey, J., Fridman, E., Pichersky, E. and Noel, J.P. (2012) Emergent decarboxylase activity and attenuation of □□ hydrolase activity during the evolution of methylketone biosynthesis in tomato. *Plant Cell* (in press).
3. Ngaki, M.N.*, Louie, G.V.*, Philippe, R.*, Manning, G., Pojer, F., Bowman, M.E., Ling, Li, Larsen, E., Syrkin Wurtele, E. and Noel, J.P. (2012) Evolution of the Chalcone Isomerase Fold from Fatty Acid-Binding to Stereospecific Enzyme. *Nature* (*equal contributions - in press).
4. Louie, G.V., Bowman, M.E., Tu, Y., Mouradov, A., Spangenberg, G. and Noel, J.P. (2010) Structure-function analyses of a Caffeic Acid O-methyltransferase from Perennial Ryegrass Reveal the Molecular Basis for Substrate Preference. *Plant Cell* 22: 4114-4127. Epub 2010 D ec 21. PubMed PMID: 21177481; PubMed Central PMCID: PMC3027180.
5. O'Maille, P.E., Malone, A., Dellas, N., Hess, B.A., Smentek, L., Sheehan, I., Greenhagen, B.T., Chappell, J., Manning, G. and Noel, J.P. (2008) Quantitative exploration of the catalytic landscape separating divergent plant sesquiterpene synthases. *Nat. Chem. Biol.* 4: 617-623. Epub 2008 Sep 7. PubMed PMID: 18776889; PubMed Central PMCID: PMC2664519.

(ii) Five Other Significant Publications

1. Dellas, N. and Noel, J.P. (2010) Mutation of Archaeal Isopentenyl Phosphate Kinase Highlights Mechanism and Guides Phosphorylation of Additional Isoprenoid Monophosphates. *ACS Chem. Biol.* 18: 589-610. PubMed PMID: 20392112; PubMed Central PMCID: PMC2887675.
2. Noel, J.P., Dellas, N., Faraldos, J.A., Zhao, Y., Hess, B.A., Smentek, L., Coates, R.M. and O'Maille, P.E. (2010) Structural Elucidation of Cisoid and Transoid Cyclization Pathways of a Sesquiterpene Synthase Using 2-Fluorofarnesyl Diphosphates. *ACS Chem. Biol.* 5: 377-392. Epub 2010 Feb 22. PubMed PMID: 20175559; PubMed Central PMCID: PMC2860371.
3. Louie, G.V., Baiga, T.J., Bowman, M.E., Koeduka, T., Taylor, J.H., Spassova, S.M., Pichersky, E. and Noel, J.P. (2007) Structure and reaction mechanism of basil eugenol synthase. *PLoS ONE.* 10: e993. PubMed PMID: 17912370; PubMed Central PMCID: PMC1991597.
4. Austin, M.B.*, Saito, T.*, Bowman, M.E., Haydock, S., Kato, A., Moore, B.S., Kay, R.R. and Noel, J.P. (2006) Biosynthesis of *Dictyostelium* discoideum differentiation-inducing factor by a hybrid type I fatty acid-type III polyketide synthase. *Nat. Chem. Biol.* 2: 494-502. Epub 2006 Aug 13. PubMed PMID: 16906151; PubMed Central PMCID: PMC2864586 (*equal contributions).
5. Austin, M.B., Bowman, M.E., Ferrer, J.-L., Schröder, J., and Noel, J.P. (2004) An Aldol Switch Discovered in Stilbene Synthases Mediates Cyclization Specificity of Type III Polyketide Synthases. *Chem. Biol.* 11: 1179-1194. PubMed PMID: 15380179.

(d) Synergistic Activities

Panel Member Metabolic Biochemistry, FIBR - National Science Foundation; Medical Student Course (UCSD) Herbal Remedies, Functional Foods, and Natural Products (11th Yr); Mentor for Salk Life Science Summer Institute program; Public Outreach Seminars through Salk's Taste of Discovery Series; Member, Science & Impact Advisory Board, John Innes Centre; Member, Scientific Advisory Board, Donald Danforth Plant Science Center; Member, Scientific Advisory Board, Max Planck Research Unit for Enzymology of Protein Folding; Member, External Advisory Committee for Pharmacology, Vanderbilt University; Editorial Advisory Board, ACS Chemical Biology; Associate Editor, The Plant Cell; Co-Founder & Chair Scientific Advisory Board, Allylix, Inc.

(e) Collaborators & Other Affiliations

(i) Collaborators and Co-Editors (past 48 months)

Michael Burkart (UCSD), Joseph Chappell (University of Kentucky), Clint Chapple (Purdue), Richard Dixon (Noble Foundation), Natalia Dudareva (Purdue), Lutz Heide (Univ. Tubingen), Rob Kay (MRC-Cambridge), Tomohisa Kuzuyama (Univ. Tokyo), Bradley Moore (UCSD), Eran Pichersky (Univ. Michigan), Joseph Schroeder (Univ. Freiburg), Vladimir Shulaev (Virginia Tech), Brenda Winkel-Shirley (Virginia Tech)

(ii) Graduate Advisors and Postdoctoral Sponsors

Ming-Daw Tsai (The Ohio State University, Ph.D. Training), Paul B. Sigler – deceased (Yale University, Postdoctoral Training)

(iii) Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Erin Bomati (Univ. Marseille), Jean-Luc Ferrer (ESRF), Joseph M. Jez (Danforth), Courtney Starks (Washington University), Chloe Zubieta (Stanford), Mark Verdecia (Oregon State Univ.).

Total Number of Postdoctoral Researches (current and past) – 8.
Total Number of Graduate Students (current and past) – 15

ADAM L OKERLUND
Translational Research Manager, NSF Engineering Research Center for Biorenewable Chemicals
Iowa State University
2134 Biorenewables Research Laboratory, Ames, IA 50011
515-294-3937/515-294-1269 (fax)/okerlund@iastate.edu

(a) Professional Preparation

Iowa State University	Chemical Engineering	BS 2002
University of Iowa	Chemical and Biochemical Engr.	PhD 2008
University of Iowa	Chemical and Biochemical Engr.	Postdoc 2008

(b) Appointments

2011 – 2012	Translational Research Manager; NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2009 – 2011	Biochemical Engineer/Sr. Research Scientist, Bioprocessing; Novus International, Inc.
2008 – 2009	Scientist; Optherion, Inc.
2008 – 2008	Postdoctoral Researcher; University of Iowa
2006 – 2006	Intern, Biochemistry; Genencor International
2002 – 2008	Graduate Student Research Assistant; University of Iowa

(c) Publications

i. Five publications most closely related to the proposed project

None

ii. Five other significant publications

Okerlund, A. L. 2008. *Naphthalene dioxygenase structure-function analysis*, PhD thesis, The University of Iowa.

Liang, H., Whited, G., Nguyen, C., Okerlund, A. & Stucky, G. D. 2008. Inherently tunable electrostatic assembly of membrane proteins. *Nano Letters,* 8**,** 333-339.

Ferraro, D. J., Okerlund, A. L., Mowers, J. C. & Ramaswamy, S. 2006. Structural basis for regioselectivity and stereoselectivity of product formation by naphthalene 1,2-dioxygenase. *Journal of Bacteriology,* 188**,** 6986-6994.

(d) Synergistic Activities

NSF Engineering Research Center for Biorenewable Chemicals – At the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) I am coordinating efforts to advance academic research into viable scalable processes.

Novus International, Inc. - At Novus International I developed several processes for metabolite purification from a complex fermentation broth. After feasibility tests and cost reduction steps were implemented, I designed and developed a scale-up procedure to increase purification levels from milligram quantities of the metabolite to kilogram quantities.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

None

ii. Graduate Advisors and Postdoctoral Sponsors

None

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

None

0 = Total number of graduate students advised and postdocs sponsored

DAVID J. OLIVER
Professor, Genetics, Development & Cell Biology
Iowa State University
229 Catt Hall, Ames, IA 50011-1301
(515) 294-4118 / (515) 294-1303 / doliver@iastate.edu

(a) Professional Preparation

Syracuse University	Biochemistry	B.S., 1971
Syracuse University	Botany	M.S., 1973
Cornell University	Plant Physiology	Ph.D., 1975
Connecticut Ag. Expt. Station		Postdoc, 1975 – 1976

(b) Appointments

1996 – present	Professor, Department of Genetics, Development & Cell Biology, Iowa State University
2003 – present	Associate Dean of Research , Iowa State University
1996 – 2003	Chair, Department of Genetics, Development & Cell Biology, Iowa State University
1989 – 1996	Professor, Biochemistry, University of Idaho
1984 – 1988	Associate Professor, Biochemistry, University of Idaho
1979 – 1983	Assistant Professor, Biochemistry, University of Idaho
1976 – 1979	Scientist, Connecticut Agricultural Experiment Station

(c) Publications

1. Nikolau, B.J., Oliver, D.J., Schnable, P.S. and Wurtele, E.S. Molecular Biology of Acetyl -CoA Metabolism. Biochem Soc Trans. **28**:591-593 (2000).

2. Behal, R.H., Lin, M. Back, S. and Oliver, D.J. Role of Acetyl-CoA Synthetase in the Leaves of *Arabidopsis thaliana*. Archives of Biochemistry and Biophysics **402** 259-267 (2002).

3. Lin, M. and Oliver, D.J. The Role of Acetyl-CoA Synthetase in Arabidopsis. Plant Physiology **147**:1822-1829 (2008).

4. Wei, Y., Lin, M. Oliver, D.J., Schnable, P.S. The Roles of Aldehyde Dehydrogenases (ALDHs) in the PDH Bypass of Arabidopsis. BMC Biochemistry 10:7 (2009)

5. Ohkama-Ohtsu, N., Oliver, D.J. Roles of γ-glutamyl transpeptidase and γ-glutamyl cyclotransferase in glutathione and glutathione-conjugate metabolism in plants. Advances in Botanical Research 52: 87-113 (2009)

6. Davletova, S., Rizhsky, L., Liang, H., Shengqiang, Z., Oliver, D.J., Coutu, J., Shulaev, V., Schlauch, K., and Mittler R. "Cross-Compartment Protection" by Cytosolic Ascorbate Peroxidase 1 (APX1) During Light Stress in Arabidopsis. The Plant Cell **17**:268-281 (2005).

7. Devi, Rama S., Chen X., Xiang, C., and Oliver, D.J. A Novel High-throughput Genetic Screen for Stress-Responsive Mutants of *Arabidopsis thaliana* Revealed New Loci Involving Stress Responses. Plant Journal **47**:652-663 (2006).

8. Lin, M. and Oliver, D.J. The Role of Acetyl-CoA Synthetase in Arabidopsis. Plant Physiology **147**:1822-1829 (2008).

9. Yu, H., Chen, X., Hong, Y.-Y., Wang, Y., Ke, S.-D., Liu, H-Y, Zhu, J-K, Oliver, D.J., and Xiang, C-B. Activated Expression of A Homeodomain-START Transcription Factor of

Arabidopsis Confers Drought Tolerance Associated with Improved Root Architecture and Reduced Stomatal Density. Plant Cell **20**:1134–1151 (2008).

10. Oliver, D.J., Nikolau, B.F., Wurtele, E.S. Acetyl-CoA – Life at the Metabolic Nexus, Plant Science 176:597-601 (2009).

(d) Synergistic Activities

As part of a multidiscipline team at the University of Idaho, I introduced an undergraduate course in "Science for Elementary Education Majors" that focused on teaching basic science principles, hands on activities, and information to this group and Native American students.
Co-PI of NSF-EPSCoR program at University of Idaho involved in preparation of grant proposal, recruiting new faculty, oversight of biological faculty, interaction with state government.
PI/co-PI of NSF-REU program at Iowa State University on "Agricultural Biotechnology" providing summer training for exceptional undergraduate and minority students.
Panel Manager for USDA Photosynthesis and Respiration Panel.
First Panel Manager for new competitive USDA Plant Biochemistry panel.
ISU-ADVANCE, Deans Council

(e) Collaborators & Other Affiliations

Collaborators (past 48 months)
Rachel Amir (Migal, Galilee Technological Center, Israel)
Abdelfattah Badr (Tanta University, Egypt)
Martin Spalding (Iowa State University, USA)

Graduate and Postdoctoral Advisors
Andre T. Jagendorf (Ph.D. Advisor), Israel Zelitch (Postdoctoral Advisor)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)
Yiming Guo (Postdoc, ISU)
Jane Roche (Postdoc, ISU)
Heather Babka (Postdoc, ISU)
Boris Eyheraguibel (Postdoc, ISU)

Past Students
Stephanie Yunkers (M.S. - Monsanto Corp.)
Kimberly Falk (Ph.D. - Max-Plank Institute, Jena, Germany)
Carol Lasko (Postdoc - Faculty, Humboldt State College)
Cecilia McIntosh (Postdoc - Faculty, East Tennessee State University)
Changlin Wang (Postdoc - Faculty, Shanghai Jiao Tong University)
Robert H. Behal (Scientist, Univ. Idaho)
Chengbin Xiang (Professor, University of Sci. and Tech. – China)
Naoko Ohtsu (Professor, Tokyo University – Japan)

Total number of graduate students advised and postdocs sponsored = 37.

ERAN PICHERSKY
Michael M. Martin Collegiate Professor
Department of Molecular, Cellular, and Developmental Biology
University of Michigan, Ann Arbor, MI 48109
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(a) Education

University of California, Berkeley	Genetics	B.Sc. 1980
University of California, Davis	Genetics	Ph.D. 1984
Rockefeller University	Molecular Biology	Post-doc, 1984-1987

(b) Appointments

2009	Visiting Professor, Australian National University, Department of Botany and Zoology, Canberra, Australia
2001-2003	Interim Chair and Chair, Department of Molecular, Cellular, and Developmental Biology (MCDB), University of Michigan
2001-present	Professor, MCDB Department, University of Michigan
2001	Visiting Professor, Hebrew University of Jerusalem, Faculty of Agriculture
2000	Visiting Alexander von Humboldt Forschungspreistrager and Senior Fulbright Scholar, Max-Planck-Institute for Chemical Ecology, Jena, Germany
1998 – 2001	Professor, Department of Biology, University of Michigan
1995 - 2000	Associate Chair for Research and Facilities, Biology Department, University of Michigan
1993	visiting Associate Professor, Institute of Biological Chemistry, Washington State University, Pullman, WA
1992 - 1998	Associate Professor, Department of Biology, University of Michigan
1986 - 1992	Assistant Professor, Department of Biology, University of Michigan

(c) Publications (*5 most closely related to work of CBiRC and 5 other significant publications*)

i. *Five publications most closely related to the proposed project*

1. Pichersky E, E Lewinsohn. Convergent evolution in plant specialized metabolism. Ann Rev Plant Biol 62:549-566 (2011).
2. Yu G, TTH Nguyen, Y Guo, I Schauvinhold, ME Auldridge, N Bhuiyan, E Fridman, Y Iijima, JP Noel, E Pichersky. The enzymatic functions of the wild tomato *Solanum habrochaites glabratum* methylketone synthases 1 and 2. Plant Physiol 154:67-77 (2010).
3. Ben-Israel I, G Yu, MB Austin, N Bhuiyan, M Auldridge, T Nguyen, I Schauvinhold, JP Noel, E Pichersky, E Fridman. Multiple biochemical and morphological factors underlie the production of methylketones in tomato trichomes. Plant Physiology 151:1952-1964 (2009).
4. Schilmiller AL, I Schauvinhold, M Larson, R Xu, AL Charbonneau, A Schmidt, C Wilkerson, RA Last, E Pichersky. Monoterpenes in the glandular trichomes of tomato are synthesized via a neryl diphosphate intermediate rather than geranyl diphosphate. Proc Natl Acad Sci USA, 106:10865-10870 (2009).
5. Schilmiller AL, RL Last, E Pichersky. Harnessing plant trichome biochemistry for the production of useful compounds. Plant J 54:702-711 (2008).

ii. *Five other significant publications*

1. Pichersky E, N. Dudareva. Scent engineering: Toward the goal of controlling how flowers smell. Trends Biotech 25:105-110 (2007).
2. Davidovich-Rikanati, R, Y Sitrit, Y Tadmor, Y Iijima, N Bilenko, E Bar, B Carmona, N Dudai, JE Simon, E Pichersky, E Lewinsohn. Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. Nature Biotech. 25:899-901 (2007).

3. Koeduka T, E Fridman, DR Gang, DG Vassão, BL Jackson, CM Kish, I Orlova, SM Spassova, NG Lewis, JP Noel, TJ Baiga, N Dudareva, E Pichersky. Eugenol and isoeugenol, charecteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. Proc Natl Acad Sci (USA) 103:10128-10133 (2006).
4. Pichersky E, JP Noel, N Dudareva. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. Science 311:808-811 (2006).
5. Fridman E, Wang J, Iijima Y, JE Froehlich, Gang DR, Ohlrogge J, E Pichersky. Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. Plant Cell 17:1252-1267 (2005).

(d) Synergistic Activities

Developed and taught for 10 years a "project lab" in plant molecular biology and biochemistry for undergraduates.

Interviewed by National Public Radio and numerous other voice and print media outlets concerning work on plant aroma biology done in my lab, with many news articles published (most recently, in the April 13, 2010 issue of the Washington Post)

Work from my lab, including an interview with me, was featured on PBS's science program, "Secrets of the Sequence".

Published an article in the lay science magazine *American Scientist* on plant volatiles.

Chaired a Gordon Conference on Plant Volatiles in 2007.

Served as Plant Biochemistry Panel Manager, AFRI, USDA, 2009.

(e) List of Collaborators, advisors, students and postdocs

i. Collaborators (past 48 months)

Cornelius Barry (Michigan State University)
Gilles Basset (University of Nebraska)
Dr. Natalia Dudareva (Purdue University)
Robert Last (Michigan State Univ)
Efraim Lewinsohn (ARO – Israel)
Joseph Noel (Salk Institute)
Rod Peakall (Australian National University, Australia)
Alexander Vainstein (Hebrew University, Israel)

ii. Graduate Advisors and Postdoctoral Sponsors

Dr. Leslie Gottlieb, UC Davis (deceased)
Post-doc Advisor: Dr. Anthony Cashmore, U. Penn (retired)
Sabbaticals with:
Dr. Rodney Croteau (Washington State University, 1993),
Dr. Jonathan Gershenzon (Max Planck Institute, Jena, 2000)
Dr. Efraim Lewinsohn (Newe Ya'ar Research center, Israel, 2001)
Rod Peakall (Australian National University, Australia, 2009)

iii. Graduate Students and Post-docs (past 4 years):

Graduate students:	*Post-docs*:	Yuki Matsuba
Adam Schmidt	Tariq Akhtar	Thuong Nguyen
Yue Yang	Nazmul Bhuiyan	Susanna Roeder
Geng Yu	Vasiliki Falara	Ines Schauvinhold
	Eyal Fridman	Goro Taguchi
	Mwafaq Ibdah	Marina Varbanova
	Yoko Iijima	Jihong Wang
	Takao Koeduka	Guodong Wang

Total number of graduate students: >10
Total number of post-docs: >25

D. RAJ RAMAN, PHD, PE
Professor and Associate Chair for Teaching, Agricultural and Biosystems Engineering
University Education Program Director and Pyrone Testbed Champion, CBiRC[1]
Iowa State University
3222 NSRIC, Ames, IA 50011-3310
(515) 294-0465 / (515) 294-4250 (fax) / rajraman@iastate.edu

(a) Professional Preparation

Rochester Institute of Technology	Electrical Engineering	B.S., 1986
Cornell University	Ag. & Biological Engineering	Ph.D., 1994

(b) Appointments

2011 - present	Associate Head for Teaching, ISU[2] ABE[3]
2010 - present	Pyrone Testbed Chamption, CBiRC
2008 – present	University Education Program Director, CBiRC
2006 – present	Assoc. Prof./Professor Agricultural & Biosystems Engineering, ISU
2006 – 2010	Assoc. Director of Educational Programs, ISU Bioeconomy Institute
2004 – 2005	Interim Head, Biosystems Engineering and Soil Science, UTK[4]
1999 – 2005	Assoc. Professor, Biosystems Engineering, UTK
1993 – 1999	Asst. Professor, Biosystems Engineering, UTK

(c) Publications

i. Five publiciations most closely related to the proposed project

1. Faulhaber, C. R., D. R. Raman, and R. T. Burns. 2011. An engineering-economic model for analyzing dairy plug-flow anaerobic digesters: cost structures and policy implications. *Transactions of the American Society of Agricultural and Biological Engineers. (In Press)*
2. Deutmeyer, A, D. R. Raman, P. T. Murphy, and S. Pandey. 2011. Effect of magnetic field on the fermentation kinetics of *Saccharomyces cerevisiae*. *Advances in Bioscience and Biotechnology* 2: 207-213.
3. Murphy, P. T., K. J. Moore, D. R. Raman, R.P. Anex, and S. L. Fales. 2011. Rapid biomass quality determination of corn stover using near infrared reflectance spectroscopy. *BioEnergy Research*. 4: 1-7
4. Rawat, V., D. R. Raman, and R. P. Anex. 2011. Technical Note: Detecting and Subcategorizing Hard-Coding Errors in Bioenergy-Relevant Spreadsheets using Visual Basic for Applications (VBA). *Applied Engineering in Agriculture* 27(3): 469-474
5. Himmelsbach, J. N., D. R. Raman, R. P. Anex, R. T. Burns, and C. R. Faulhaber. 2010. Effect of ammonia soaking pretreatment and enzyme addition on biochemical methane potential of switchgrass. *Transactions of the ASABE.* 53(6):1921-1927

ii. Five other significant publications

1. Cruse, M. J., M. Z. Liebman, D. R. Raman, M. H. Wiedenhoeft. Fossil Energy Use in Conventional and Low-External-Input Cropping Systems. *Agronomy Journal* 102(3):934-941
2. Himmelsbach, J. N., A. Isci, D. R. Raman, and R. P. Anex. 2009. Design and Testing of a Pilot-Scale Aqueous Ammonia Soaking Biomass Pretreatment System, *Applied Engineering in Agriculture* 25(6): 953-959
3. Haney, L. J., J. G. Coors, A. J. Lorenz, D. R. Raman, R. P. Anex, and M. P. Scott. 2008. Development of a fluorescence-based method for monitoring glucose catabolism and its potential use in a biomass hydrolysis assay. *Biotechnology for Biofuels* 2008, **1**:17
4. Isci, A., J. N. Himmelsbach, J. Strohl, A. L. Pometto III, D. R. Raman, and R. P. Anex. 2008. Pilot Scale Fermentation of Aqueous Ammonia Soaked Switchgrass, *Applied Biochemistry and Biotechnology* published online 21 Aug 2008, DOI 10.1007/s12010-008-8235-y.

[1] NSF Engineering Research Center for Biorenewable Chemicals
[2] Iowa State University
[3] Agricultural and Biosystems Engineering
[4] The University of Tennessee, Knoxville

5. Isci, A., J. N. Himmelsbach, A. L. Pometto III, D. R. Raman, and R. P. Anex, 2007. Aqueous Ammonia Treatment of Switchgrass Followed by Simultaneous Saccharification and Fermentation, *Applied Biochemistry and Biotechnology* 144(1): 69–77.

(d) Synergistic Activities

1. CBiRC REU Creator/Director
2. SBPP REU Co-Director
3. CenUSA Educational Programs Co-Director
4. Associate Chair for Teaching, ABE – entails both BSE program creator and lead recruiter/accreditation specialist and A E program oversight
5. Mentor to ASABE Student Club and new BSE student club

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Rob Anex	University of Wisconsin	Adah Leshem-Ackerman	Iowa State University
Thomas Bobik	Iowa State University	Matthew Liebman	Iowa State University
Robert Burns	University of Tennessee	Aaron Lorenz	University of Wisconsin
Katrina Christiansen	Cargill	Thomas Lubberstedt	Iowa State University
Michael Cruse	University of Wisconsin	Ronald Madl	Kansas State University
Nancy Da Silva	University of California, Irvine	Melissa Montalbo-Lomboy	Iowa State University
Abhaya Datye	University of New Mexico	Kenneth Moore	Iowa State University
Robert Davis	University of Virginia	Patrick Murphy	Purdue University
Alex Deutmeyer	Garmin	Matthew Neurock	University of Virginia
Julie Dickerson	Iowa State University	Basil Nikolau	Iowa State University
James Dumesic	University of Wisconsin	Joe Noel	Salk Institute
Lars Dunn	Unknown	David Oliver	Iowa State University
Steven Fales	Iowa State University	Santosh Pandey	Iowa State University
Carol Faulhaber	Nestle	Eran Pichersky	University of Michigan
Ramon Gonzalez	Rice University	Andrew Proctor	University of Arkansas
David Grewell	Iowa State University	Vertika Rawat	No current affiliation
Lisa Haney	Pioneer	Peter Reilly	Iowa State University
Jennifer Himmelsbach	Merck	Derek Rollins	Iowa State University
Guiping Hu	Iowa State University	Ka-Yiu San	Rice University
Asli Isci	Ankara University	Suzanne Sandmeyer	Univ. of California, Irvine
Darren Jarboe	Iowa State University	Marvin (Paul) Scott	Iowa State University
Laura Jarboe	Iowa State University	Brent Shanks	Iowa State University
Lawrence Johnson	Iowa State University	Jackie Shanks	Iowa State University
Peter Keeling	Iowa State University	Mary Wiedenhoeft	Iowa State University
George Kraus	Iowa State University	Keith Woo	Iowa State University
Kendal Lamkey	Iowa State University	Eve Wurtele	Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors; (ISU=Iowa State University)

Joshua Claypool, MS, ISU; Darren Jarboe, PhD, ISU; Carol Faulhaber, MS, Nestle, Inc.; Vertika Rawat, MS, No current affiliation; Katrina Christiansen, PhD, Cargill; Patrick Murphy, PhD, Purdue Univ.; Jenni Himmelsbach, MS, Merck; Jasjeet Kaur, MS, Texas A&M, currently a PhD

iii. Thesis Advisor and Postgraduate-Scholar Sponsors (past 48 months) (ISU=Iowa State University)

Jeremiah Johnson, MS, ISU, Agric. Engr.; Xuan Li, MS, ISU, Agric. Engr.; Joseph Vanstrom, PhD, ISU, Industrial & Agric. Technology; Steve Sell, MS, ISU, Agric. Engr.; Pedro Ortiz, PhD, ISU, Chemical Engr.; Alex Deutmeyer, MS, ISU, Electrical Engr.; Cody Ellens, MS, ISU, Mechanical Engr.; Gang Sun, PhD, ISU, Agric. Engr.; Jared Brown, MS, ISU, Mechanical Engr.; Michael Cruse, MS, ISU, Agronomy; Jason Haegele, MS, ISU, Agronomy; Katherine Edwards, MS, ISU, Biorenewable Resources Tech.; Melissa Montalbo-Lomboy, PhD, ISU, Biorenewable Resources, Tech.; Neslihan Akdeniz, PhD, ISU, Agric. Engr.; Steve Bly, MS, ISU, Agric. Engr.; Tim Shepherd, MS, ISU, Agric. Engr.; Wei Wu, MS, ISU, Agric. Engr.; Asli Isci, PhD, ISU, Agric. Engr.

26 = Total number of graduate students advised and postdocs sponsored.

PETER J. REILLY
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515-294-5968 / 515-294-2689 (fax) / E-mail address: reilly@iastate.edu

(a) Professional Preparation

Princeton University	Chemistry	A.B., 1960
University of Pennsylvania	Chemical Engineering	Ph.D., 1964

(b) Appointments

2005 – Present	Professor of Chemical and Biological Engineering, ISU
1992 – Present	Anson Marston Distinguished Professor in Engineering, ISU
1979 – 2005	Professor of Chemical Engineering, ISU
1974 – 1979	Associate Professor of Chemical Engineering, ISU
1968 – 1974	Assistant Professor of Chemical Engineering, Univ. of Nebraska
1964 – 1968	Research Engineer, E. I. du Pont de Nemours & Company, Deepwater, NJ

(c) Publications

i. Five publications most closely related to the proposed project

1. Johnson, G. P., L. Petersen, A. D. French, and P. J. Reilly. Twisting of Glycosidic Bonds by Hydrolases. *Carbohydr. Res*., **344**, 2157 (2009).
2. Warner, C. D., J. A. Hoy, T. C. Shilling, M. J. Linnen, N. D. Ginder, C. F. Ford, R. B. Honzatko, and P. 2. J. Reilly. Tertiary Structure and Characterization of a Glycoside Hydrolase Family 44 Endoglucanase from *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.*, **76**, 338 (2010).
3. Petersen, L., A. Ardèvol, C. Rovira, and P. J. Reilly. Molecular Mechanism of the Glycosylation Step Catalyzed by Golgi a-Mannosidase II. A QM/MM Metadynamics Investigation. *J. Am. Chem. Soc.,* **132**, 8291 (2010).
4. Cantu, D. C., Y. Chen, and P. J. Reilly. Thioesterases: A New Perspective Based on Their Primary and Tertiary Structures. *Protein Sci.,* **19**, 1281 (2010).
5. Barker, I. J., L. Petersen, and P. J. Reilly. Mechanism of Xylobiose Hydrolysis by GH43 b-Xylosidase. *J. Phys. Chem. B,* **114**, 15389 (2010).

ii. Five other significant publications

1. Warner, C. D., R. M. Go, C. García-Salinas, C. Ford, and P. J. Reilly. Kinetic Characterization of a Glycoside Hydrolase Family 44 Endoglucanase from *Ruminococcus flavefaciens* FD-1. *Enzyme Microb. Technol*., **48**, 27 (2011).
2. Cantu, D. C., Y. Chen, M. L. Lemons, and P. J. Reilly. ThYme: A Database for Thioester-Active Enzymes. *Nucleic Acids Res.,* **39**, D342 (2011).
3. Jing, F., D C. Cantu, J. Tvaruzkova, J. P. Chipman, B. J. Nikolau, M. D. Yandeau-Nelson, and P. J. Reilly. Phylogenetic and Experimental Characterization of an Acyl-ACP Thioesterase Family Reveals Significant Diversity in Enzymatic Specificity and Activity. *BMC Biochem.*, **12**, 44 (2011).
4. Chen, Y., E. E. Kelly, R. P. Masluk, C. L. Nelson, D. C. Cantu, and P. J. Reilly. Structural Classification and Properties of Ketoacyl Synthases. *Protein Sci.*, **20**, 1659 (2011).

5. Hill, A. D., and P. J. Reilly. Scoring Functions for AutoDock. *Molecular Methods in Biochemistry*. M. Frank and T. Lüttke, eds. Part III – Structural Bioinformatics. Springer. Accepted for publication.

(d) Synergistic Activities

Speakers' Bureau member, American Chemical Society, 1984–2009 (68 sections visited)
Speakers' Bureau member, American Institute of Chemical Engineers, 1987–2000 (27 sections visited)
Advisor, Iowa State University Chapter, Society of Hispanic Professional Engineers, 1986–1992
Coordinator, Iowa State University–University of Glasgow Exchange, 1984–2002; Iowa State University–Université de Lausanne–Ecole Polytechnique Fédérale de Lausanne Exchange, 1985–present
Since 1999, my refereed publicatons have had 22 different undergraduate coauthors; nine of those papers have had undergraduate first authors.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Ardevol, Albert	University of Barcelona, Spain
Ford, Clark	Iowa State University
French, Alfred	USDA Southern Regional Research Center
Fushinobu, Shinya	University of Tokyo, Japan
Ginder, Nathaniel	Washington University Medical School
Gu, Xun	Iowa State University
Hidaka, Masafumi	National Food Research Institute, Japan
Honzatko, Richard	Iowa State University
Hoy, Julie	University of Guelph, Canada
Johnson, Glenn	University of Iowa
Kitaoka, Motomitsu	National Food Research Institute, Japan
Lemons, Matthew	Iowa State University
Nerinckx, Wim	Ghent University, Belgium
Nikolau, Basil	Iowa State University
Rovira, Carme	University of Barcelona, Spain
Yandeau-Nelson, Marna	Iowa State University

ii. Graduate and Postdoctoral Advisors (your own)

Arthur E. Humphrey	Retired

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Cantu, David	Iowa State University
Chen, Yingfei	Iowa State University
Hill, Anthony	St. Jude Medical Center, Minneapolis
Mertz, Blake	University of Arizona, Tucson, Ariz.
Peterson, Luis	OPKO Pharmaceutical Co., Guadalajara, Mexico
Shilling, Taran	Houston, Tex.
Vander Velden, Kent	Pioneer Hi-Bred Seeds, Johnston, Ia.
Warner, Christopher	Scripps Research Institute, Jupiter, Fla.

57 = Total number of graduate students advised and postdocs sponsored.

DERRICK K. ROLLINS, SR.
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515-294-5516. / 515-294-2689. / drollins@iastate.edu

(a) Professional Preparation

Kansas University	Chemical Engineering	BS 1979
The Ohio State University	Chemical Engineering	MS 1987
The Ohio State University	Statistics	MS 1979
The Ohio State University	Chemical Engineering	PhD 1990

(b) Appointments

1/1/10 – Present	Professor-In-Charge of Community Based Recruiting and Transition, College of Engineering
1/1/08 – 1/1/10	Assistant Dean, College of Engineering
7/1/07 – Present	Professor, Chemical and Biological Engineering, Statistics
7/1/95-6/30/07	Associate Professor, Chemical and Biological Engineering, Statistics
8/15/90 – 6/30/95	Assistant Professor, Chemical and Biological Engineering, Statistics

(c) Publications

1. Rollins, D. K. and A. Teh, “An extended data mining method for identifying differentially expressed assay-specific signatures in functional genomic studies,” BioData Mining 2010, 3:11.
2. Rollins, D. K., N. Bhandari, J. Kleinedler, K. Kotz, A. Strohbehn, L. Boland, M. Murphy, D. Andre, N. Vyas, G.Welk and W. Franke, "Free-living inferential modeling of blood glucose level using only noninvasive inputs," Journal of Process Control 20 95-107 (2010).
3. Rollins, D.K., K. Kotz and C. Stiehl, “Non-invasive Glucose Monitoring From Measured Inputs,” Proceeding of the UKACC International Conference on CONTROL 2010 7-10 September, Coventry, UK.
4. Zhai, D., D. K. Rollins, and N. Bhandari, "Block-oriented Continuous-time Modeling or Nonlinear Systems under Sinusoidal Inputs," the International Journal of Modelling and Simulation, **28**(2) (2008).
5. Rollins, D. K. and G. L. Larson, "Estimating a Minimum Set of Physically-Based Dynamic Parameters to Enhance Statistical Inference in Block-Oriented Modeling," Computers and Chemical Engineering **32** 494-502 (2008).
6. Rollins, D. K., D. J. Rollins and A. D. Jones, "Spatial-Temporal Semi-empirical Dynamic Modeling of Thermal Gradient CVI Processes," Chemical Engineering Research and Design **85**(A10) 1390-1396 (2007).
7. Hardjasamudra, A., D. K. Rollins, N. Bhandari, and S. Chin, "Optimal Experimental Design for Wiener Systems," Chemical Engineering Communications **194**, 656-666 (2007).
8. Rollins, D. K, D. Zhai, A. L. Joe, J. W. Guidarelli, and R. Gonzalez, "A Novel Data Mining Method to Identify Assay-Specific Signatures in Functional Genomic Studies," BMC Bioinformatics, **7** 377 (2006).
9. Hulting, S., D. K. Rollins, and N. Bhandari,"Optimal Experimental Design for Human Thermoregulatory System Identification," Chemical Engineering Research and Design **84**(A11), 1-10 (2006).

10. Zhai, D., H. Wu., N. Bhandari, and D. K. Rollins, "Continuous-Time Hammerstein and Wiener Modeling Under Second Order Static Nonlinearity for Periodic Process Signals," Computers & Chemical Engineering, **31**, 1-12 (2006).

(d) Synergistic Activities

Developed and taught three-day short course "Probability and Statistical Inference for Chemical Engineering Faculty and Graduate Students"; Developed and taught three-day industrial short course "Time Series Methodologies for the Process Control Engineer," at 3m; Developed a pioneering non-residential summer enrichment programs in math, physics and literature for raising minority ninth (2001), tenth (2002), and eleven (2003) graders in the Des Moines School System as part of the ISU Science Bound Program; 2001 organizing committee and speaker at the NSF workshop: Minority ChE Faculty 2001+: A Workshop to Develop Minority Leaders in the ChE Academy; Diversity Advisor to the ISU President's Cabinet since1996.

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (past 48 months)

Nsarg Vyas	BodyMedia, Inc.
Dale Seborg	University of California, Santa Barbara
Frank Doyle	University of California, Santa Barbara
Ali Cinar	Illinois Institute of Technology
Eric Brey	Illinois Institute of Technology
Dale Wesson	Florida A&M University

Graduate Advisor:
Jim Davis (UCLA)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 Months)

Graduate Students:

• Ai-ling Teh (M.S. Statistics – 2010)	Seeking employment in Malaysia
• Amanda Bell (M.S. Statistics – 2010)	US Census Bureau
• Emmanuel Criner (M.S. Statistics – 2009)	Unknown
• Stephanie Loveland (Ph.D. ChE -- 2008)	Senior Lecturer, CBE Department, ISU
• Swee-teng Chin (Ph.D. ChE -- 2007)	Dow Chemical Company
• William Rodriquez (M.E. ChE – 2006)	Dow Chemical Company
• Gabrielle Larson (M.S. Stat, Fall 2006)	Industry

Current Students: Lucas Beverlin, Kaylee Kotz

Postdoctoral Researchers (last 48 Months)
- Nidhi Bhandari (Visiting professor, 2007-current)
- Cory Stiehl (Post Doc, 2008-)

40 = Total number of graduate students advised and postdocs sponsored

KA-YIU SAN
Professor, Department of Bioengineering
Rice Unviersity
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Houston, Texas 77005
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(a) Professional Preparation

Rice University	Chemical Engineering	B.S., 1978
California Institute of Technology	Chemical Engineering	M.S., 1981
California Institute of Technology	Chemical Engineering	Ph.D., 1984
California Institute of Technology	Biochemical Engineering	Jan. 1984 – July 1984

(b) Appointments

2004-present	E.D. Butch Professor in Bioengineering, Rice University
1996-2004	Professor, Bioengineering, Rice University
1996-present	Professor, Chemical and Biomolecular Engineering, Rice University
1990-1996	Associate Professor, Chemical Engineering, Rice University
1984-1990	Assistant Professor, Chemical Engineering, Rice University

(c) Publications

i. Five publications most closely related to the proposed project

1. Martínez, I., Zhu, J., Lin, H., Bennett, G.N., San, K.-Y., " Replacing Escherichia coli NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with a NADP-dependent enzyme from *Clostridium acetobutylicum* facilitates NADPH dependent pathways, Metabolic Engineering, 10(6):352-359 (2008).
2. Sánchez, A. M., Bennett, G. N., and San, K.-Y., Novel pathway engineering design of the anaerobic central metabolic pathway in *Escherichia coli* to increase succinate yield and productivity, Metabolic Engineering, 7(3):229-239 (2005).
3. Zhang X, Li M, Argawal A, San K-Y. Efficient free fatty acid production in *Escherichia coli* using plant acyl-ACP thioesterases. Metabolic Engineering, 13:713-722 (2011).
4. Zhang X, Argawal A, San K-Y. Improving fatty acid production in *Escherichia coli* through the overexpression of malonyl coA-Acyl carrier protein transacylase. Biotechnology Progress, 28:60-65 (2012).
5. Thakker C, Martínez I, San K-Y, Bennett GN. Succinate production in *Escherichia coli*. Biotechnol J. 7:213-224 (2012).

ii. Five other significant publications

1. Zhu, J., Shalel Levanon, S., Bennett, G. N., and San, K.-Y., The YfiD protein contributes to the pyruvate formate-lyase flux in an *E. coli* arcA mutant strain, Biotechnology and Bioengineering, 97:138-43 (2007).
2. Shalel-Levanon, S., San, K.-Y., and Bennett, G. N., Effect of oxygen on the *E. coli* ArcA and FNR regulation systems and metabolic responses, Biotechnology and Bioengineering, 89:556-64 (2005).
3. Sánchez, A. M., Bennett, G. N., and San, K.-Y., Batch culture characterization and metabolic flux analysis of succinate producing *E. coli* strains, Metabolic Engineering, 8:209–226 (2006).
4. Peebles CAM, Sander GW, Hughes EH, Peacock R, Shanks JV, San K-Y. The expression of 1-deoxy-D-xylulose synthase and geraniol 10-hydroxylase or anthranilate synthase increases

terpenoid indole alkaloid accumulation in *Catharanthus roseus* hairy roots. Metabolic Engineering, 13:234-40 (2011).
5. Zhu J, Sanchez A, San K-Y, Bennett GN. Manipulating Respiratory Levels in *Escherichia coli* for Aerobic Formation of Reduced Chemical Products. Metabolic Engineering, 13:704-712 (2011).

(d) Synergistic Activities

Co-author of a textbook, "Bioengineering Fundamentals", with Ann Saterbak and Larry V. McIntire (Prentice Hall, January 2007). The textbook is intended for sophomore level bioengineering introductory course. The book emphasizes on the quantitative treatment of conservation principles using biological systems as examples.
Involved in teaching a course in biochemical engineering;
Many strains of bacteria and plasmids constructed in the lab have been sent to colleagues throughout the world.
Currently serve on the editorial board of journals in the area of biochemical and metabolic engineering.

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Ateeque Ahmad	Konkuk University, S. Korea
George N. Bennett	Rice University
Ill-Min Chung	Konkuk University, S. Korea
Sue Gibson	University of Minnesota
Ramon Gonzalez	Rice University
Kathleen Mathews	Rice University
Jackie V. Shanks	Iowa State
Jackie V. Shanks	Iowa State
PraveenV. Vadlani	Kansas State University
Kyriacos Zygourakis	Rice University

ii. Graduate and Postdoctoral Advisors

Gregory N. Stephanopoulos	California Institute of Technology (Graduate advisor)
Gregory N. Stephanopoulos	California Institute of Technology (Postdoctoral advisor)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)*

Stephanie Porter	Glycos Biotechnologies
Christie Peebles	Colorado State University
Irene Martinez	Pontifical Catholic University of Valparaiso, Chile
Joanna Jan	Current, Rice
SongI Han	Current, Rice
Jiangfeng Zhu*	Chinese Academy of Sciences, Qingdao Inst. of Bioenergy and Bioprocess
Mathew Wong*	Glycos Biotechnologies
Grant Blazer*	NREL
Leepika Tuli*	Current, Rice
Mai Li*	Glycos Biotechnologies
Yane Luo*	Current, Rice
Hui Wu*	Current, Rice
Xuijun Zhang*	Current, Rice

13 = graduate students advised and postdocs sponsored.

SUZANNE B. SANDMEYER
Professor, Biological Chemistry and Microbiology & Molecular Genetics
Associate Director, UCI Institute for Genomics and Bioinformatics
Director, Protein and DNA Mircoarray Facility
University of California, Irvine
Dept. of Biological Chemistry, D240 Med Sci I, Irvine, CA 92697-1700
(949) 824-7571/(949) 824-2688 (fax)/sbsandme@uci.edu

(a) Professional Preparation

Carleton College	Biology	B.A., 1973
University of Washington	Biochemistry	Ph.D., 1980
Washington University	Genetics	Postdoc, 1980 – 1982

(b) Appointments

2011-present	Professor, Chemical Engineering and Materials Science, School of Engineering
2009 – present	Associate Director, UCI Institute for Genomics & Bioinformatics, University of California, Irvine (UC-Irvine)
2000 – present	Director, Genomics High-Throughput Facility, UC-Irvine
1997 – present	Professor, Biological Chemistry, UC-Irvine (primary appointment)
1997 – 2005	Chair, Dept. of Biological Chemistry, UC-Irvine
1994 – present	Professor, Microbiology & Molecular Genetics, UC-Irvine
1990 – 1994	Assoc. Professor, Microbiology & Molecular Genetics, UC-Irvine
1984 – 1990	Asst. Professor, Microbiology & Molecular Genetics, UC-Irvine
1982 – 1983	Research Associate, Genetics, Washington University, St. Louis, MO
1974 – 1980	Research Associate, Biochemistry University of Washington, Seattle, WA
1973 – 1974	Teaching Assistant, Biochemistry, University of Washington, Seattle, WA

(c) Publications

i. Five publications most closely related to the proposed project (yeast vectors, bioengineering, and retrotrotransposon strain modifications)

1. Qi, X., Daily, K., Nguyen, K., Wang, H., Mayhew, D., Rigor, P., Forouzan,S., Johnston, M., Mitra, R.D., Baldi, P. and **Sandmeyer, S.B.** (2012) Retrotransposon profiling of RNA polymerase III initiation sties Retrotransposon profiling of RNA polymerase III initiation sites. Genome Res. Online Jan 27, 2012, doi:10.1101/gr.131219.111.
2. Fang, F., Salmon, K., Shen, M., Aeling, K, Ito, E., Irwin, B., Tran, U., Hatfield, G.W., Da Silva, N.*, and Sandmeyer, S.* (2011) A combinatorial vector set for metabolic engineering in *Saccharomyces cerevisiae*. Yeast 28:123-136.
3. Clemens, K., Larsen, L.Z., Zhang, M., Kuznetsov, Y., Bilanchone, V., Beliakova-Bethell, N., Randall, A., DaSilva, R., Nagashima, K., McPherson, A., Baldi, P. and **Sandmeyer, S.B.** (2011) Ty3 spacer controls intracellular condensation and uncoating. J. Virol. 85, 3055-3066.
4. Irwin, B., Aye, M., Baldi, P., Beliakova-Bethell, N., Cheng, H., Dou, Y., Liou, W. and Sandmeyer, S.B. 2005. Retroviruses and yeast retrotransposons use overlapping sets of host genes. Gen. Res. **15**:641-654.
5. Beliakova-Bethell, N., Beckham, C., Giddings, T.H. Jr., Winey, M., Parker, R., and Sandmeyer, S. (2006) Virus-like particles of the Ty3 retrotransposon assemble in association with P-body components. RNA **12**:94-101

ii. Five other significant publications (yeast molecular biology and biochemistry)

1. Beliakova-Bethell, N., Terry, L.J., Bilanchone, V.W., DaSilva, R., Nagashima, K., Wente, S., and **Sandmeyer, S.B.** (2009). Ty3 nuclear entry is initiated by viruslike particle docking on nucleoporins. J. Virol. J. Virol. 83: 11914-11925. *Chosen for Nov Issue Spotlights.*
2. Larsen, L.S.Z., Beliakova-Bethell, N., Bilanchone,V., Zhang, M., Lamsa, A., DaSilva, R., Hatfield, G.W., Nagashima, K., and Sandmeyer, S.B. (2008). Ty3 nucleocapsid controls localization of particle assembly. J. Virol. 82:2501-2514

3. Kuznetsov, Y.G., Zhang, M., Menees, T. McPherson, A**,** and Sandmeyer, S. (2005) Atomic force microscopy investigation of the structure of Ty3 retrotransposon particles. J. Virol. **79**: 8032-8045.
4. Aye, M. Irwin, B., Archibald, H., and Sandmeyer, S.B. (2004) Host factors that affect Ty3 retrotransposition in *Saccharomyces cerevisiae*. Genetics 168, 1159-1176.
5. Sandmeyer, S., Aye, M., and Menees, T. (2002) Ty3: A Position-Specific, Gypsylike Element in *Saccharomyces cerevisiae.* In *Mobile DNA,* ASM Press, Washington DC.

(d) Synergistic Activities

Lecturer in Explore Science on Saturdays Program [NSF FOCUS-Faculty Outreach Collaborations Uniting Scientists, Students and Schools (https://eee.uci.edu/11w/05450/eSOS2011/)] weekend lectures to students attending community colleges serving populations under-represented in the sciences.
Organization of undergraduate laboratory to investigate molecular biology of *Yarrowia*, an oleaginous yeast
Associate Director of Institute for Genomics and Bioinformatics (recipient of NLM training grant for bioinformatics and campus intercisciplinary research unit for bioinformatics)
Director of the UCI Genomics High-Throughput Facility (1999-present).
Chair, Department of Biological Chemistry (1997-2005).
Co-Chair of Cold Spring Harbor Retroviruses Meeting (2003).
Member, National Cancer Institute, Division of Basic Sciences, Board of Scientific Counselors (1998-2003)
Chair, Senior Editors, *Genetics* (2006-2007).
Organizer, UCI Mini-Symposia: Mobile DNA 02/19/10; 02/2011 HTS to P4 Medicine

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

P. Baldi (U.C. Irvine); J. Briggs, EMBL, Heidelberg, Germany; N. DaSilva (UCI); G.W. Hatfield (UCI); L. Huang (UCI); M. Johnston (U. CO, Denver); R. Lathrop (U.C. Irvine); A. McPherson (U.C. Irvine); R. Mitra (Washington U., St. Louis, MO); K. Nagashima (SAIC, NCI Frederick); B. Shanks (ISU).

ii. Graduate and Postdoctoral Advisors

Postdoctoral Advisor: Maynard Olson, (U WA, Seattle, WA)
Ph.D. Thesis Advisor: Paul Bornstein, (U WA, Seattle, WA)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor

Ph.D. --Lori Hansen, Director, Program and Alliance Management at Seattle Genetics; Douglas Chalker, Associate Professor, Dept Biology, Washington University; Philip Kinsey, DNA Technical Leader, State of MT; Jacqueline Kirchner, Project Manager, Amgen, Seattle, WA; Charles Connolly, Research Staff, University of Washington; Kathryn Orlinsky, homemaker; Jonathan Claypool, Patent attorney, Claypool Intellectual Property, Seattle, WA ; Jirong Gu, Product Manager for BioSolutions, Varian, Inc. Irvine, CA; Sophia Lin, MD/PhD, Pediatric Physician, Children's Hospital Orange County; Lynn Yieh, Bioinformatician, Johnson and Johnson, San Diego, CA; Henrietta Nymark, Invitrogen Inc., San Diego, CA, now homemaker; Liza Zicker-Larsen (2002-2006 Staff Scientist, CODA Genomics Inc., Laguna Hills, CA); Min Zhang (2002-2007) postdoctoral fellow in virology, UCLA; Nadia Beliokova-Bethell, Postdoctoral fellow, Professor Christopher Woelk, UCSD, San Diego, CA; Michael Aye, Ph.D. Postdoctoral Fellow, David Allis, U. VA; Liza Zicker-Larsen (Postdoc, 2007 Staff Scientist Verdezyne, Inc., Carlsbad, CA); Kim Nguyen (2004-2010) Postdoctoral Fellow; Daniel Voytas, U. MN
Past postdoctoral fellows--Virginia Bilanchone, Project scientist, UCI, Biological Chemistry*;* Douglas Forrest, Staff Scientist, US FDA*;* Thomas Menees, Professor, University of MO, Kansas City, MO*;* Joerg Hoffman, Senior Scientist, Division of Biochemistry, Friedrich-Alexander University Erlangen-Nurnberg Sandra Dildine, unknown; Victoria Perreau, Senior Research Fellow, Centre for Neuroscience, U of Melbourne, Australia; Michael Aye, Project Manager, Focus Diagnostics, Irvine, CA; Fang Fang, Genetic Scientist, EdeniQ Inc, Goshen, CA; Tarek Najdi, Bioinformatician, British Petroleum, San Diego, CA
Current Students--Kristina Christiansen *;* James Yu
Current Postdoctoral Associates: Xiaojie Qi*;* Jicheng Duan *;* Ivan Chang

Total number of graduate students advised and postdocs sponsored (including present) = 31.

KLAUS SCHMIDT-ROHR
Professor, Dept. of Chemistry, Iowa State University, Ames, IA 50011
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(a) Professional Preparation

Heidelberg & Mainz Universities, Germany	Physics	Diploma, 1989
Mainz University/Max-Planck Institute for Polymer Research, Germany	Physics	Ph.D., 1991
Multidimensional NMR Methods for the Investigation of Dynamics, Structure, & Order in Solid Polymers.		
University of California at Berkeley	Physical Chemistry	1993 – 1994

(b) Appointments

2005-present	Full Professor, Dept. of Chemistry, Iowa State University, Ames, IA
2000-2004	Associate Professor, Dept. of Chemistry, Iowa State University, Ames, IA
1997–1999	Associate Professor with tenure, Dept. of Polymer Science and Engineering, University of Massachusetts at Amherst
1995–1997	Assistant Professor, Dept. of Polymer Science and Engineering, UMass Amherst
1993–1994	Postdoctoral Research Fellow, Dept. of Chemistry, UC Berkeley, with A. Pines
1992	Project Manager, Max-Planck Institute (MPI) for Polymer Research
1991 – 1992	Staff Scientist, MPI for Polymer Research, laboratory of H. W. Spiess

(c) Publications

i. Five publications most closely related to the proposed project

1. C. E. Brewer, Y-Y. Hu, K. Schmidt-Rohr, S. D. Joseph, T. E. Loynachan, R. C. Brown, "Characteristics of the extent of pyrolysis for corn stover fast pyrolysis biochars", *J. Environm. Quality*, in press (2011).
2. C. E. Brewer, K. Schmidt-Rohr, J. A. Satrio, R. C. Brown, "Characterization of Biochar from Fast Pyrolysis and Gasification Systems" *Environ. Progr. Sust. Energy* **28**, 386-396 (2009).
3. X-W. Fang , T. Chua, K. Schmidt-Rohr, M. L. Thompson**,** "Quantitative ^{13}C NMR of Whole and Fractionated Iowa Mollisols for Assessment of Organic Matter Composition", *Geochim. Cosmochim. Acta* **74**, 584-598 (2010).
4. J-D. Mao, K. Schmidt-Rohr, "Accurate Quantification of Aromaticity and Nonprotonated Aromatic Carbon Fraction in Natural Organic Matter by ^{13}C Solid State Nuclear Magnetic Resonance", *Environ. Sci. Technol*. **38**, 2680-2684 (2004).
5. J-D. Mao, K. Schmidt-Rohr, "Recoupled Long-Range C-H Dipolar Dephasing in Solid-State NMR, and Its Use for Spectral Selection of Fused Aromatic Rings", *J. Magn. Reson.* **162**, 217-227 (2003).

ii. Five other significant publications

1. X-W. Fang, K. Schmidt-Rohr, "**Alkyl and Other Major Structures Formed in Model Maillard Reactions Studied by Solid-State NMR**"**,** *J. Agri. Food Chem.* **59**, 481-490 (2011).
2. Y.-Y. Hu, A. Rawal, K. Schmidt-Rohr, "Strongly bound citrate stabilizes the apatite nanocrystals in bone", *Proc. Natl. Acad. Sci. USA* **107**, 22425-22429 (2010).
3. J-D. Mao, X-W. Fang, Y. Lan, A. Schimmelmann, M. Mastalerz, L. Xu, K. Schmidt-Rohr, "Chemical and nanometer structure of kerogen and its change during thermal maturation investigated by advanced solid-state ^{13}C NMR spectroscopy", *Geochim. Cosmochim. Acta* **74**, 2110-2127 (2010).

4. J.-D. Mao, K. M. Holtman, J. T. Scott, J. F. Kadla, K. Schmidt-Rohr, "Differences between Lignin in Unprocessed Wood, Milled Wood, Mutant Wood, and Extracted Lignin Detected by ^{13}C Solid-State NMR", *J. Agri. Food Chem.* **54**, 9677-9686 (2006).
5. K. Schmidt-Rohr, J-D Mao, D.C. Olk, "Nitrogen-Bonded Aromatics in Soil Organic Matter and Their Implications for a Yield Decline in Intensive Rice Cropping", *Proc. Nat. Acad. Sci.* **101**, 6351-6354 (2004).

(d) Synergistic Activities

- Associate Editor of *Organic Geochemistry*, 2002-2005
- Member of the Editorial Boards of *Polymer* and *Solid State NMR*

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

M. Akinc (Iowa State), R. C. Brown (Iowa State); B.F. Chmelka (UC Santa Barbara); B. C. Cook (Ames Lab), J. P. Heremans (Ohio State), M. Hong (Iowa State), M. G. Kanatzidis (Northwestern), D. A. Laird (ISU), J. Lehmann (Cornell), S. Mallapragada (Iowa State), J-D. Mao (Old Dominion), M. Mastalerz (Indiana), D. M. McKnight (CO-Boulder), R. B. Moore (Virgina Tech), K. Müllen (MPI Mainz), D. C. Olk (Natl. Soil Tilth Lab), J. Otaigbe (USMiss), A. Schimmelmann (Indiana), R. Venkatasubramanian (RTI), B. H. Shanks (ISU), H. W. Spiess (MPI Mainz), M. L. Thompson (Iowa State)

ii. Graduate Advisor and Postdoctoral Sponsors

Professor Hans W. Spiess, Max-Planck Institute for Polymer Research, Mainz, Germany
Postgraduate advisor: Professor Alexander Pines, Dept. of Chemistry, University of California, Berkeley CA

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Graduate Students:
Current (2): Mr. Robert L. Johnson; Ms. Jinfang Cui
Past (12): Mr. Wei-Guo Hu (1999), Mr. Ioannis S. Polios (1997), Mr. Matthew G. Dunbar (1999), Mr. Douglas J. Harris (1999), Mr. Mikhail Y. Gelfer (1999), Mr. Daniel Mowery (2002, Florida State), Ms. Gabriele Menges (2003), Mr. Qiang Chen (2005; UNC), Mr. Aditya Rawal (2007; Ames Lab), Mrs. Xiaowen Fang (2008; UW Madison), Mr. Xueqian Kong (2010; UC Berkeley), Ms. Yanyan Hu (2011; Stony Brook)
Past Visiting (3): Mr. Kay Saalwächter, Mr. Eduardo R. deAzevedo, Mr. Fábio Becker-Guédes
Postdoctoral Associates /Visiting Scientists (10):
Dick Sandström (Stockholm), Kristin K. Kumashiro (Hawaii), Hironori Kaji (Kyoto, Japan), Tito J. Bonagamba (Sao Carlos, Brazil), Jingdong Mao (Rocky Mountain College), Detlef Reichert (Halle, Germany), Shengshu Hou (Nat'l Cheng Kung Univ., Taiwan), Evgenii Levin (Ames Lab), Mr. Aditya Rawal (Ames Lab); Mrs. Bosiljka Njegic (Ames Lab)

JACQUELINE V. SHANKS
Manley R. Hoppe Professor, Chemical & Biological Engineering Department
Iowa State University
4136 Biorenewables Research Laboratory, Ames, IA 50011-2230
(515) 294-4828/(515) 294-2689 (fax)/jshanks@iastate.edu

(a) Professional Preparation

Iowa State University	Chemical Engineering	B.S., 1983
California Institute of Technology	Chemical Engineering	Ph.D., 1989

(b) Appointments

2010-present	Thrust 2 Leader, NSF Center for Biorenewable Chemicals (CBiRC), ISU
2009-present	Manley R. Hoppe Professor, Department of Chemical and Biological Engineering, Iowa State University
2008–2010	Thrust 2 Co-Leader, NSF Center for Biorenewable Chemicals (CBiRC), ISU Professor, Department of Chemical and Biological Engineering, Iowa State University
1999 – present	Adjunct Professor, Department of Bioengineering, Rice University
1999 – 2009	Professor, Chemical Engineering, Iowa State University
1999	Professor, Bioengineering and Chemical Engineering, Rice University
1997 – 1999	Associate Professor, Bioengineering, Rice University
1993 – 1999	Associate Professor, Chemical Engineering, Rice University
1988 – 1993	Assistant Professor, Chemical Engineering, Rice University

(c) Publications (Selected from 80 peer-reviewed publications)

i. Five publications most closely related to the proposed project

1. Choudhary, M.K., Yoon, J.M., Gonzalez, R. and J.V. Shanks. 2011. Metabolic Fluxes of *Escherichia coli* in Anaerobic Fermentation of Glucose Using 2-dimensional Nuclear Magnetic Resonance (NMR) Spectroscopy. *Biotechnology and Bioprocess Engineering* ***16,*** *419-437.*
2. Peebles, C.A.M., Sander, G. W., Hughes, E. H., Peacock, R., J.V. Shanks, and K.-Y. San.2011. The Expression of 1-deoxy-D-xylulose Synthase and Geraniol 10-hydroxylase or Anthranilate Synthase Increases Terpenoid Indole Alkaloid Accumulation in *Catharanthus roseus* Hairy Roots. *Metabolic Engineering* **13**, 234-240. http://dx.doi.org/10.1016/j.ymben.2010.11.005
3. Murarka, A., Clomburg, J. M., Moran, S., Shanks, J. V. and R. Gonzalez. 2010. Metabolic Analysis of Wild-Type *Escherichia coli* and a Pyruvate Dehydrogenase Complex (PDHC)-deficient derivative reveals the Role of PDHC in the Fermentative Metabolism of Glucose. *J. Biological Chemistry*,**285**, 31548-31558. doi:10.1074/jbc.M110.121095
4. Iyer, V.V., Sriram, G., Fulton, D. B., Zhou, R., Westgate, M.E., and Shanks, J.V. 2008. Metabolic Flux Maps Comparing the Effect of Temperature on Protein and Oil Biosynthesis in Developing Soybean Cotyledons. *Plant Cell and Environment*, 31 (4), 506–517. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.2008.01781.x/pdf
5. Sriram, G., D. B. Fulton, and Shanks, J. V. 2007. Flux Quantification in Central Carbon Metabolism of *Catharanthus roseus* Hairy Roots by ^{13}C Labeling and Comprehensive Bondomer Balancing. *Phytochemistry,* **68**, 2243-2257. *http://dx.doi.org/10.1016/j.phytochem.2007.04.009*

ii. Five other significant publications

1. Sriram, G., Iyer, V. V., Fulton, D. B., and Shanks, J. V. 2007. Identification of Hexose Hydrolysis Products in Metabolic Flux Analytes: A Case Study of Levulinic acid in Plant Protein Hydrolysate. *Metabolic Engineering,* **9**, 442-451.
2. Sriram, G.,González-Rivera, O. and Shanks, J. V. 2006. Determination of Biomass Composition of *Catharanthus roseus* Hairy Roots for Metabolic Flux Analysis, *Biotech. Progress,* **22,** 1659 – 1663**.** DOI: 10.1021/bp060162k
3. Sriram, G., Fulton, D. B., Iyer V., Peterson, J. M., Zhou, R., Westgate, M. E., Spalding, M. H. and Shanks, J. V. 2004. Quantification of Compartmented Metabolic Fluxes in Developing Soybean (*Glycine max*) Embryos by Employing Biosynthetically Directed Fractional ^{13}C Labeling, 2-D [^{13}C, ^{1}H] NMR and Comprehensive Isotopomer Balancing. *Plant Physiol.* **136**: 3043-3057. www.plantphysiol.org/cgi/doi/10.1104/pp.104.050625
4. Sriram, G.and Shanks, J. V. 2004. Improvements in Metabolic Flux Analysis using Carbon Bond Labeling Experiments: Bondomer Balancing and Boolean Function Mapping. *Metabol. Eng,* **6**, 116-132. doi:10.1016/j.ymben.2004.02.003
5. Morgan, J. and Shanks, J. V. 2002. Quantification of Metabolic Flux in Plant Secondary Metabolism by a Biogenetic Organizational Approach. *Metabolic Engineering*, **4**, 257-262.

(d) Synergistic Activities

Thrust 2 Leader, NSF Center for Biorenewable Chemicals (CBiRC), Iowa State University
DOE, The Office of Biological and Environmental Research (BER) Advisory Committee (BERAC) 2011- present
AIChE Food, Pharmaceutical and Bioengineering Division, Area 15c Plenary Award 2010
Editorial Board: *Biotechnology Progress*, *Current Opinion in Biotechnology*
Mentoring of over 60 undergraduate and high school researchers in engineering – of which over 65% are women and underrepresented minorities, (1988-present)
Mentoring of 2 female graduate students in Mech. Engr; of 3 CBE assistant professors (2 female, 1 URM)

(e) Collaborators & Other Affiliations (outside ISU)

i. Collaborators and Co-Editors (past 48 months)

Nancy DaSilva, Chemical Engineering, University of California Irvine; John Everard, Dupont, Delaware Sue Gibson, Plant Biology, University of Minnesota; Ramon Gonzalez, Chemical and Biomolecular Engineering, Rice University; Harin Kanani, Pioneer Hybrid International; Costas Maranas, Penn State University; Govind S. Nadathur, Marine Sciences, University of Puerto Rico, Mayaguez; Ka-Yiu San, Bioengineering, Rice University; Suzanne Sandmeyer, Biological Chemistry, University of California Irvine; Gordon V. Wolfe, Biological Sciences, California State University, Chico ; Larry Wackett, Biochemistry, University of Minnesota

ii. Graduate Advisor

James E. Bailey (deceased)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 60 months)

Past Students (last 5 years and current position):

Madhuresh Choudhury (M.S. 2008, Indo-Gulf Fertilizer); Vidya Iyer (Ph.D. 2006, Bristol Myers Squibb) Guy Sander (Ph.D. 2009, Asst. Professor, Univ. of Minn. Duluth); Yanfen Fu (M.S. 2011, pre-doctoral student, Univ. of Washington)

Current Students:

Le Zhao, Mark Brown, Ting Wei Tee, Quyen Truong, Erin Boggess, BCB (co-advised by Julie Dickerson)

Current Research Scientist

Jong Moon Yoon

Total number of graduate students advised and postdocs sponsored = 30.

L. KEITH WOO
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(a) Professional Preparation

Harvey Mudd College	Chemistry	B.S., 1977
Stanford University	Chemistry	Ph.D., 1984
University of Wisconsin-Madison	Chemistry	1984-1986

(b) Appointments

2004-present	Associate Chair, Department of Chemistry
2003-present	Professor, Department of Chemistry, Iowa State University
1992-2003	Associate Professor, Department of Chemistry, Iowa State University
1986-1992	Assistant Professor, Department of Chemistry, Iowa State University

(c) Publications

i. Five publications most closely related to the proposed project

1. Bagherzadeh, M.; Amini, M.; Ellern, A.; Woo, L. K. "Palladium and copper complexes with oxygen–nitrogen mixed donors as efficient catalysts for the Heck reaction." *Inorg. Chim. Acta*, **2012**, *383*, 46-51. http://dx.doi.org/10.1016/j.ica.2011.10.040
2. Klobukowski, E. R.; Angelici, R. J.; Woo, L. K. "Bulk Gold-Catalyzed Reactions of Isocyanides, Amines, and Amine N-Oxides." *Organometallics* **2012**, *31*, In press. http://dx.doi.org/10.1021/om201068g
3. Klobukowski, E. R.; Angelici, R. J.; Woo, L. K. "Bulk Gold Catalyzed Oxidations of Amines and Benzyl Alcohol Using Amine N-Oxides as Oxidants." *Catal. Lett.* **2012**, *142*, 161-167. http://dx.doi.org/10.1007/s10562-011-0758-0
4. Bagherzadeh, M.; Amini, M.; Ellern, A.; Woo, L. K. "Catalytic Efficiency of a Novel Complex of Oxoperoxo Molybdenum(VI): Synthesis, X-ray Structure and Alkane Oxidation." *Inorg. Chem. Commun*. **2012**, *15*, 52-55. http://dx.doi.org/10.1016/j.inoche.2011.09.037
5. Klobukowski, E. R.; Mueller, M.; Angelici, Woo, L. K. "Conversions of Cyclic Amines to Nylon Precursor Lactams Using Bulk Gold and Fumed Silica Catalysts." *ACS Catalysis*, **2011**, *1*, 703-708. http://dx.doi.org/10.1021/cs200120c

ii. Five other significant publications

1. Zhou, Y.; Angelici, R. J.; Woo, L. K. "Bulk Gold-Catalyzed Reactions of Diazoalkanes with Amines and O_2 to Give Enamines." *Catal. Lett.* **2010**, *137*, 8-15. http://dx.doi.org/10.1007/s10562-010-0339-7
2. Mbuvi, H. M.; Klobukowski, E. R.; Roberts, G.; Woo, L. K. "O-H Insertion and Tandem N-H Insertion/Cyclization Reactions Using an Iron Porphyrin as Catalyst with Diazo Compounds as Carbene Sources." *J. Porphyr. Phthalocya.* **2010**, *14*, 284-292. http://dx.doi.org/10.1142/S1088424610001982
3. Zhou, Y.; Trewyn, B. G.; Angelici, R. J.; Woo, L. K. "Catalytic Reactions of Carbene Precursors on Bulk Gold Metal." *J. Am. Chem. Soc.*, *J. Am. Chem. Soc.* **2009**, *131*, 11734-11743. http://dx.doi.org/ 10.1021/ja900653s

4. Mbuvi, H; Woo, L. K. "Catalytic C–H Insertions Using Iron(III) Porphyrin Complexes." *Organometallics*, **2008**, *27*, 637-645. http://dx.doi.org/10.1021/om7007502
5. Zhou, Y.; Ryu, E.-H.; Zhao, Y.; Woo, L. K. "Solvent Responsive Metalloporphyrins: Binding and Catalysis." *Organometallics*, 2007, *26*, 358-364. http://dx.doi.org/10.1021/om060791z

(d) Synergistic Activities

1. Mentor to NSF REU undergraduate researchers
2. Senior Personnel in NSF ERC (2008-2012)
3. Mentor to NSF RET middle school teachers
4. Member of Faculty Advisory Board of ISU Bioeconomy Institute
5. HHMI participant for transforming undergraduate teaching laboratories

(e) Collaborators & Other Affiliations

i. Collaborators and Co-Editors (past 48 months)

Robert J. Angelici	Iowa State University
Andrew Hillier	Iowa State University
Yan Zhao	Iowa State University

ii. Graduate Advisors and Postdoctoral Sponsors

Charles P. Casey	University of Wisconsin-Madison
James P. Collman	Stanford University

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

BJ Anding	Iowa State University
Mojtaba Bagherzadeh	Visiting Professor, Sharif University of Technology, Iran
Taiwo Dairo	Iowa State University
Erik Klobukowski	Ph.D., Iowa State University
Harun M. Mbuvi	Ph.D., Iowa State University
Gina Roberts	Iowa State University
Yibo Zhou	Postdoc, Iowa State University

35 = Total number of graduate students advised and postdocs sponsored

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(a) Professional Preparation

University of California, Santa Cruz	Biology	B.S., 1971
University of California, Los Angeles	Biology	Ph.D., 1980
University of California, Davis	Biochemistry	Postdoc, 1980 – 1983

(b) Appointments

1999 – present	Professor, Genetics, Development & Cell Biology, Iowa State University
1995 – 1999	Associate Professor, Botany, Iowa State University
1990 – 1995	Assistant Professor, Botany, Iowa State University
1983 – 1988	Senior Research Scientist, Cell Biology Division, NPI, Inc.

(c) Publications

i. Five publications most closely related to the proposed project

1. Ngaki MN, Louie GV, RN, Manning G, Pojer F, Bowman ME, Li L, Elise Larsen E, Wurtele ES, Noel JP. 2012. Evolution of the Chalcone Isomerase Fold from Fatty Acid-Binding to Stereospecific Enzyme. Nature.. In press, March
2. Schneller W, Campbell PJ, Bassham D, **Wurtele ES** 2012. Meta!Blast computer game: a pipeline from science to 3D art to education. Proc. SPIE 8289, 828905 http://dx.doi.org/10.1117/12.911289
3. Feng YP, Hurst J. Almeida-De-Macedo M. Chen X. Li L. Ransom N, **Wurtele ES**. 2012. A massive human co-expression-network and its medical applications. Summit on Systems Biology, Chemistry & Biodiversity. *In Press*
4. Quanbeck SMM, et al., Welti R, Ilarslan H, **Wurtele ES**, Nikolau .BJ. 2012. Metabolomics as a hypothesis-generating functional genomics tool for the annotation of Arabidopsis thaliana genes of "unknown function" Frontiers of. Plant Science. doi: 10.3389/fpls.2012.00015

ii. Five other significant publications

1. Peng J, Ilarslan H, **Wurtele ES**, Bassham DC. 2011. AtRabD2b and AtRabD2c have overlapping functions in pollen development and pollen tube growth. BMC Plant Biology. 11:25
2. Stenerson M, Schneller W, **Wurtele ES**. 2012. Enabling Educators to Customize the Game Environment. Games, Learning and Society Conference, 2011. Abstract at: glsconference.org/2011/program/event/73
3. Li X, Ilarslan H, Brachova L, Qian HR, Li L, Che P, **Wurtele ES**, Nikolau BJ. 2011 Reverse genetic analysis of the two biotin-containing subunit genes of the heteromeric acetyl-CoA carboxylase in Arabidopsis thaliana indicates a unidirectional functional redundancy. Plant Physiol. PubMed PMID: 21030508.
4. Sucaet Y, **Wurtele ES**. 2010. MetNetAPI: A flexible method to access and manipulate biological network data from MetNet. BMC Res Notes. 3:312. PubMed PMID: 21083943; PubMed Central PMCID: PMC2998519.
5. Wu L, Rowe EW, Jeftinija K, Jeftinija S, Rizshsky L, Nikolau BJ, McKay J, Kohut M, **Wurtele ES.** 2010. Echinacea-induced cytosolic Ca2+ elevation in HEK293. BMC Complement Altern Med.10:72. PubMed PMID: 21092239; PubMed Central PMCID: PMC3002894.

(d) Synergistic Activities

Co-Organizer, **Third International Congress on Plant Metabolomics**, PSI Symposium, June, 2004, Iowa State University. Supported in part by awards from NSF and USDA. (275 attendees, more than half of these were international participants).

Organizer, **Metabolic Networking in Plants**, April, 1999, First in a series of international symposia on Plant Molecular Biology and Biochemistry at Iowa State University. Supported in part by awards from NSF and USDA.

Editor, BMC Plant Biology

Panel/Study Section member:

National Science Foundation: Metabolic Biochemistry (six panels): Interagency Metabolic Engineering: Systems Biology (one panel); Arabidopsis 2010 (one panel)

National Institute of Health: Modeling and Analysis of Biological Systems (study section, 3 years), Science Education Partnership Association (study section, 1 year)

USDA: Plant Biochemistry (two panels)

Multinational Arabidopsis Steering Committee: Subcommittee on Systems Biology.

International Advisory Board for Academic Freedom, Bar Ilan University.

Mentor for high school and undergraduate interns, and high school teachers in: Howard Hughes Initiative Foundation, Women in Science and Engineering, NSF-REU (eight to ten students/year), NSF-RET (one teacher/year).

Meta!Blast Awards

2012 Featured in *Science, Feb 2012:* International SciVis Challenge: Winner, Honorable Mention, Interactive Videogame. National Science Foundation

2012 Featured by NSF at American Association for Advancement of Science Annual Meeting

2012 International SciVis Challenge Finalist, Illustration (The Cytosol).

2010 Finalist Learning Lab, (top 5% of >1000 submissions) MacArthur Foundation,

2010 Second Place, Chlorofilms.

MUSEUM EXHIBITS: Meta!Blast

- Birla Science Center, Hyderabad, India. Winter 2009

Museums of the National Council of Science Museums of India http://www.ncsm.org.in/

- Birla Industrial & Technological Museum, Kolkata (Headquarters)
- Biotechnology Gallery of Visvesvaraya Industrial & Technological Museum, Bangalore,
- Regional Science Centre, Guwahati, Assam
- Nehru Science Centre Mumbai
- Local NCSM science centers at Kalimpong, Gangtok, Siliguri

(e) Collaborators & Other Affiliations

i. Collaborators (past 48 months)

Bai, J. (ISU), Che, Ping (ASU), Myers, Alan (ISU), Nielsen-Hamilton, M. (ISU), Ohlrogge, John (Michigan State Univ.), Oliver, D. J. (ISU), Reinot, Andres (ISU), Rhee, Sue (Stanford Univ.), Schnable, Patrick (ISU), Shanks, Jackie (ISU), Spalding, Martin (ISU)), Sumner, L. W. (Nobel Foundation),

ii. Graduate and Postdoctoral Advisors

Bernard Phinney (UCLA)deceased, Eric Conn (UC-Davis)

iii. Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Heather Babka, Suh-Yeon Choi, Matthew Hillwig, Jie Li, Li Ling, Lankun Wu, Hailong Zhang, Micheline Ngaki, Yves Sucaet, Jon Hurst, Yaping Weng, Matt Crispin, Dallas Jones

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